The application of multivariate analysis to aid interpretation of textile fibre dyes analysed by microspectrophotometry

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The application of multivariate analysis to aid interpretation of textile fibre dyes analysed by microspectrophotometry

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Abstract

There have been numerous recent publications calling for an increase in the reliability of forensic evidence. Furthermore, there have been comments on a noticeable lack of research published with regard to the application of multivariate analysis to textile fibre evidence.

In this work a classification system was proposed that would utilise a probabilistic approach, require minimal user input, and be robust. The system utilised microspectrophotometry data collected from various fibres - without the use of additional analytical techniques such as microscopy or thin layer chromatography to represent a more streamlined and objective approach. A set of optimal settings for the classification system were established through experimentation utilising acrylic and cotton fibres from indistinguishable and distinguishable sources. In addition, two multivariate analysis approaches were investigated; the application of principal component analysis for dimension reduction followed by linear discriminant analysis (PCA-LDA) and the utilisation of linear discriminant analysis alone (LDA-own). The optimal settings for the proposed classification system were found to be upper/lower self-predictive probability (SPP) = 0.9999/0.0001, exceedance proportion (EP) = 0.5 and number of fibres per group = 10.

Up to 100% classification accuracy was observed when considering both fibres from indistinguishable and distinguishable sources – provided that 10 fibres were available from both sources and that the dye composition of both sources were suitably dissimilar if they were truly from different sources. If only single fibres were available for analysis, or the dye composition between truly different sources of fibres was too similar then classification accuracy decreased.

Outline of Thesis

Chapter 1 – “Introduction” – Outlines some key foundational information around topics such as: fibres, dyeing of fibres, light and colour, textile analysis in forensic science, microspectrophotometry (MSP) and multivariate analysis (MVA).

Chapter 2 – “Materials and Methods” – Outlines the approach utilised for sampling, mounting and examining fibres using MSP before covering the statistical techniques utilised and providing information relating to the validation of the standard operating procedure (SOP) utilised in this research.

Chapter 3 – “Developing an Ideal Classification System” – Outlines the rationale behind the research design and choice/application of the MVA techniques proposed.

Chapter 4 – “Establishing the Optimal Settings for the Classification System“- Covers a series of experiments whereby the optimal settings for the MVA approach are established using a range of acrylic and cotton fibres from various sources. These settings are then applied in the subsequent sections.

Chapter 5 – “The Application of Multivariate Analysis to Colour Block Scenarios” – Applies the previously established optimal settings to comparisons of groups of red cottons, blue cottons and black cottons as three of the most common colour/fibre combinations encountered in forensic science.

Chapter 6 – “Limits of Discrimination and Single Fibre Scenarios” – Examines the sensitivity of the proposed methodology by utilising cotton samples of known dye concentrations and proportions dyed in house; before investigating the classification accuracy of proposed methodology when only a single fibre is available for analysis.

Chapter 7 – “Conclusions” – Outlines the main conclusions of this research.

Chapter 8 – “Glossary” – contains brief definitions of key terms.

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Undertaking this research and completing this thesis has been the hardest task I have encountered in my life. The entire process has seen a massive reduction in my mental and physical health; to the point where at one time it looked like this research would not reach its conclusion or ever be submitted. Everything contained within is the accumulation of years of hard work and struggle.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted by the Faculty Ethics Committee (RE-CFS-024) on 6th November 2014.

I declare that the Word Count of this Thesis is approximately 40000 words

Name: Rory Kenneth Simmons

Signature:

Date: 15/1/2021

1. Introduction

1.1 Fibres, Light and Dyeing

1.1.1 Textile Fibres

1.1.1.1 Introduction

Textile fibres are an important form of trace evidence encountered at crime scenes. The transfer of textile fibres can link people, tools, and crime scenes by inferring contact between two individuals, or between individuals and objects [1]. The situation of fibre evidence in Germany and the UK was discussed at the European Textile and Hair Group (ETHG) (A European Network of Forensic Science Institutes (ENFSI) working group) meeting in 2017 where it was reported that the "surplus value of fibre evidence, although undisputed among forensic scientists, is not always recognized by police and legal representatives" [2].

A textile fibre is a unit of matter, either natural or synthetic, that forms the basic element of fabrics and other textiles [3]. Both natural and synthetic fibres are originally opaque and colourants, i.e. dyes or pigments, are added to them to make them commercially useful [4]. Colour is an important characteristic of fibre evidence and its analysis is necessary to allow for successful inclusion or exclusion of textile fibres [1,3]. The colour that is observed on a fibre is due to the reflectance from dye(s) applied on the fibre.

Previous studies [5–11] have shown synthetic fibres to be highly polymorphic and may vary in features such as cross-sectional shape, diameter, delustrant concentrations and distribution, birefringence etc., but colour also continues to play an important role [4]. For certain fibres, such as cotton, the main characteristic for discrimination is colour [1] since these fibres tend to be much less uniform in structure, cross sectional shape etc. than synthetic fibres such as

acrylic and polyester [4]. This combined with their high usage, means that some natural fibres may be perceived to have reduced evidential value compared to synthetic fibres [4].

There are six dominant fibre types found worldwide today: cotton, wool, polyester, polyamide (nylon), acrylic and viscose [4,12]. This research will heavily utilise cotton fibres and as such cotton will receive the greatest detail in the following sections. Polyester, as the most commonly produced and utilised synthetic fibre [13] and acrylic as the other fibre type investigated are also discussed in this section.

1.1.1.2 Natural Fibres

Natural fibres, with the exception of silk, exist as staple fibres with lengths typically ranging from 2 cm to 50 cm in length and need to be spun into yarn. The objective of yarn manufacture is to bring together a series of short parallel fibres and, by using frictional forces created by insertion of twists, hold the bundles of fibres together in a strong continuous length [4]. Synthetic fibres and silk are produced as continuous filament yarns that can be cut to specific lengths depending on their end use [4].

A natural fibre is defined by Houck and Siegel as “any fibre that exists as a fibre in its natural state” and a synthetic fibre as “any fibre derived by a process of manufacture from any substance which, at any point in the manufacturing process, is not a fibre” [3]. Natural fibres fall into one of three classes: cellulose based (from seeds, stems and leaves of plants) e.g. cotton, protein based (from hair, wool or silk of animals), and mineral based (e.g. asbestos) [4].

The most common fibre worldwide and in forensic laboratories is typically white cotton, whose sources are so numerous and fibres from different sources indistinguishable – meaning they have little value as forensic evidence as there is no colour component (dye) to analyse and morphological feature comparison has poor discriminating power for natural fibres [4]. Cotton fibres have a natural twist or convolution which is present during fibre growth [4]. Cotton comes from the seedpod of plants in the genus Gossypium and is grown in subtropical climates [4]. In 2009, Gordon [14] reported that two species are commonly cultivated: G. hirsutum (upland cotton) which accounted for ~94% of world production and G. barbadense (Egyptian cotton) which accounted for ~4% of world production.

The principle component in cotton is cellulose, the polysaccharide macro molecule which makes up a plant's cell wall, and makes approximately 92-95% of the makeup of cotton; with the remainder being made up of hemicellulose, pectin, fat/wax and lignin in decreasing amounts [12]. These ratios can be altered by processes such as scouring (which removes waxes and pectins); thus increasing the proportions of all other components [4]. The structure of cellulose is shown below in Figure 1.

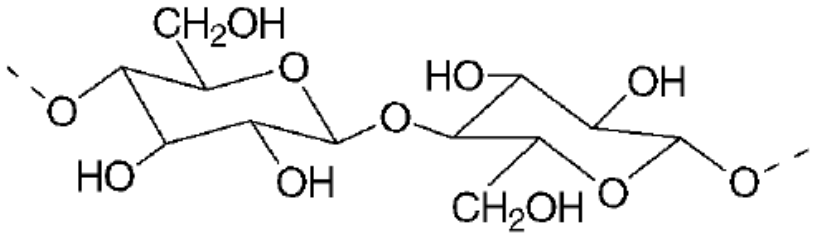

Figure 1: The structure of cellulose [12]

Cellulose has a fairly open structure which allows large dye molecules to penetrate into the fibre relatively easily. Within the long chains of repeating glucose units, each glucose unit contains three hydroxyl groups – two of which are secondary and one primary. These make the cellulose molecule relatively polar. The ability of the hydroxyl groups to form intermolecular hydrogen bonds is of importance in direct dyeing [12].

1.1.1.3 Synthetic Fibres

Similarly to natural fibres, synthetic fibres also fall into three classes: regenerated fibres (from naturally occurring fibre-forming polymers e.g. viscose from cellulose), synthetic fibres (from non-renewable sources e.g. polyester) and inorganic fibres (formed from inorganic materials such as glass) [4].

Fibres from non-renewable sources (i.e. synthetic fibres) are manufactured from one of two polymer types; condensation polymers and addition polymers. Condensation polymers (e.g. polyester) are prepared from the condensation reaction of two monomers having two functional groups from which a simple molecule is eliminated. Addition polymers (e.g. acrylic) are formed by the direct addition of the monomer to itself without the elimination of any molecules and must contain double bonds for polymerisation [4].

The first synthetic fibre produced was nylon in 1939 [4]. Increases in population growth and consumerism since World War II have led to an increase in synthetic fibre production as natural fibres alone would not have been able to meet demand [4]. Synthetic fibres can be characterised by a number of morphological variables including: cross-sectional shape, diameter, delustrant concentration & distribution

and birefringence – giving them a high degree of variability even before considering dyes or colour [4,6–11,15] as opposed to natural fibres. This combined with their wide use, means natural fibres tend to have reduced evidential value compared to synthetic fibres.

Synthetic fibres are produced through “spinning” – whereby fibres are formed by extruding a fibre forming substance (the spinning dope) through hole(s) in a showerhead-like device - a spinneret. The spinning dope is created by rending solid monomeric material into a liquid or semi-liquid form with a solvent or heat [3].

There are three methods of spinning: melt spinning, dry spinning and wet spinning. In melt spinning, molten polymer is forced through the holes of the spinneret and filaments are formed as the polymer cools and, as such, melt spinning can only be applied to thermoplastic polymers. In dry spinning, the polymer is dissolved in a volatile organic solvent and the resulting viscous solution is pumped through the spinneret into an air column where filaments are formed as the solvent evaporates. Wet spinning involves the polymer being in solution (either aqueous or organic solvent) and being pumped through the spinneret into a bath of coagulating chemical which causes precipitation [4]. Following extrusion, filaments are stretched or drawn mechanically to orientate the molecular chains along the longitudinal axis of the fibre; maximising the molecular forces between the molecular chains and increasing polymer crystallinity and fibre strength [4].

Considering acrylic specifically, as acrylic is utilised in this research, there are two types of acrylic fibres, acrylic and modacrylic; both polymerised from acrylonitrile. Acrylic fibres contain at least 85% (by weight) of acrylonitrile whereas modacrylic contains 35-85% acrylonitrile. The polymerisation of acrylonitrile is initiated by free

radicals, is exothermic and can be either by wet or dry spinning [4]. Acrylonitrile is often polymerised in conjunction with a co-monomer such as methacrylic acid or methyl acrylate - which is used to open up the molecular structure and incorporate anionic or cationic groups, thus increasing dyeability of the fibre [4].

Acrylic fibres are normally dyed with modified basic (cationic) dyes through the process of gel dyeing, whereby the acrylic tow while in gel form is passed through a dyebath containing the cationic dye - yielding bright shades with excellent light and wet fastness [4]. The high bulk characteristics of acrylic make it highly suitable for the knitwear industry, whereas modacrylic is often blended with other fibres to enhance their flame retardant properties before using in home furnishings, curtains etc.

1.1.1.4 Fibres Worldwide

In 2015, Houck and Siegel stated over half of the fibres produced each year are natural fibres, and the majority of these are cotton and that approximately half of all fibres produced annually are cotton [3]. In 2016, Lepot, De Wael and Lunstroot [16] reported that worldwide production of synthetic fibres increased from 25 million tons in 65 million tons (166% increase) between 1994 and 2014 - this has gone hand in hand with a decrease in cotton's domination of the worldwide market share [17].

Amongst synthetic fibres, polyester maintained a lead in worldwide production, owning 76% of the population share in 2014 [17]. Over the same period, the share of other synthetic fibres such as acrylic and nylon have decreased [16]. Citing the 2018 European Man-made Fibre Association Report [18], Lepot, Lunstroot and De

Wael report that synthetic fibres continue to see increased production while cotton and wool shares decrease. They reported that synthetic fibres represented 75% of all textile fibres being produced worldwide (81% in Europe) [13].

The U.S. Department of Agriculture estimated for 2018/19 that worldwide cotton production was down 1% on the previous season, and that overall lower harvest areas were expected – but that global yield was expected the be the fifth highest on record and the overall cotton consumption was set to expand by 3.8% [19]. Similarly, in 2018, Koszewska stated they predict cotton and polyester production to grow 40% globally over the following 5 years [20]. Despite this increase in synthetic fibre production and application, many of the published population studies published prior to 2015 [6–11,15,21–24] have shown synthetic fibres formed a low percentage (<20%) of any population studied.

1.1.2 Light and Colour

1.1.2.1 Wavelengths of the electromagnetic spectrum

Visible light refers to the region of the electromagnetic spectrum to which human eyes are sensitive - within the wavelength range of approximately 360 nm to 780 nm [1,12]. White light contains the entire wavelength range outlined above, although not necessarily in equal quantities [12]. Below this region (i.e. < 360 nm) is the ultraviolet (UV) region and above it (i.e. > 780nm) the infrared (IR) range.

The visible spectrum is made up of wavelengths of lights that can be recognised by the human eye in terms of colour; with examples of wavelength ranges, absorbed wavelengths and observed colours being shown below in Table 1. When an object absorbs light of a particular wavelength/colour, a complimentary colour

corresponding to the remaining wavelengths of incident light that have not been absorbed is observed by the eye [12]. For example, an object that absorbs green light (500-560 nm) will appear purple because the blue and red components are transmitted or reflected.

Table 1: Colour Absorbed vs. Colour Observed by Eye [4]

Wavelength range (nm)	Colour absorbed	Colour observed by human eye
380-430	Violet	Green-yellow
430-480	Blue	Yellow
480-490	Green-blue	Orange
490-500	Blue-green	Red
500-560	Green	Purple
560-580	Yellow-green	Violet
580-590	Yellow	Blue
590-610	Orange	Green-blue
610-750	Red	Blue-green

Light at the lower end of the visible spectrum has a longer wavelength of about 780 nm, and is seen as red, violet is at the upper end of the spectrum having a wavelength of about 360 nm, and green-yellow is approximately in the middle [1]. These wavelengths and the colours observed are shown below in Figure 2.

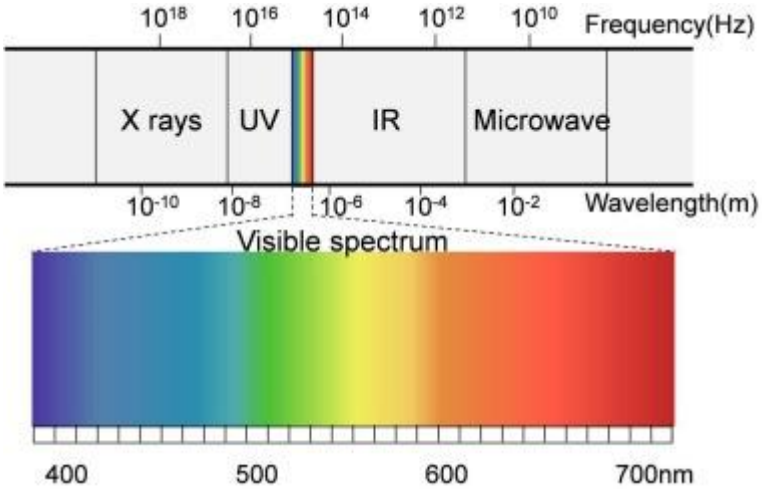

Figure 2: The wavelengths and colours in the visible spectrum [1]

However, the colours response of the eye is based upon the varying sensitivity of cone cells – meaning that the visual descriptions of colours are subjective [1]. This issue of subjective colour interpretation, and therefore comparison of colour, is further discussed later in this research.

1.1.2.2 Interactions between the electromagnetic spectrum and matter

Interactions between the ultraviolet and visible (UV-vis) region of the electromagnetic spectrum and matter can be better understood by considering the wavelike characteristics of radiation. An electron becomes excited if the frequency, which is related to wavelength, of the incident electromagnetic radiation matches or closely corresponds to the difference in energy between two electronic states. This leads to absorption – whereby resonance excitation leads to a change in electron density distribution and, ultimately, an electronic transmission from the highest occupied molecular orbital to the lowest unoccupied molecular orbital [4].

Two of the most important ways light can interact with an object, with respect to desired observed colour, are absorption and scattering. Absorption is the process

by which radiant energy is utilised to raise molecules to higher energy states and scattering is the interaction by which light is re-directed as a result of multiple refractions and reflections [12]. If only absorption is involved when light interacts with an object it will appear transparent as any light not absorbed is transmitted through the object. If scattering of light occurs, light will be reflected back to the observer and the object will appear translucent or opaque [12].

A dye in solution owes its colour to the selective absorption of certain wavelengths of light by dye molecules; with the remaining wavelengths of light being transmitted and giving rise to the observed colour [12]. The absorption of light energy by the dye molecule promotes electrons in the molecule from a low energy state (ground state) to a higher energy state (excited state) – termed an electronic transition. This energy difference between the two states, ΔE, is given by Planck's relationship (Equation 1) – where *h* is Planck's constant, *c* is the velocity of light (constant) and λ is the wavelength of light absorbed (nm). Thus, there is an inverse relationship between ΔE and the wavelength of light it absorbs [12].

Equation 1: Planck's relationship [12]

$$\Delta E = \frac{hc}{\lambda}$$

1.1.2.3 Colour

Colour is often described using three attributes: shade (hue), intensity and brightness [12]. Colours can be mixed to create other colours and there are two ways this can be achieved: "additive" and "subtractive" mixing. Additive mixing refers to the mixing of coloured lights so that the source of illumination is observed

by the eye Red, blue and green are the primary colours used through additive mixing. Subtractive mixing is when colours are observed as a result of reflection from, or transmission through, an object – after interaction with incident white light. Yellow, magenta and cyan are the primary colours used with subtractive colour mixing. The colours described previously in Table 1 that are observed due to selective light absorption are known as chromatic colours, and form the basis of subtractive colour mixing [12]. Subtractive colour mixing is involved when dyes (and pigments) are mixed, and is therefore the process involved with analysis of fibre dyes using microspectrophotometry (MSP) which is discussed in more depth in the MSP section.

Colour may be introduced to manufactured articles for a variety of reasons, but amongst the most common purpose is to enhance the appearance and attractiveness of a product and improve its market appeal [12]. The colour differences between fibres may be very small and indistinguishable to the naked eye – thus the need for objective and sensitive methods such as MSP. For many fibres, including cotton, the only characteristic that can be reliably used for discrimination is colour [1].

Christie [12] states that there are fifteen causes of colour arising from a variety of physical and chemical mechanisms that can be categorised into five groups (a-e below), and that industrially important organic dyes deal almost exclusively with colour generated by the mechanisms described in group c.

a) Colour from simple excitations, vibrations and rotations

b) Colour from ligand effects, metal compounds and metal impurities

c) Colour from molecular orbitals, organic compounds and charge transfer

d) Colour from band theory, metals, semiconductors and doped semiconductors and colour centres.

e) Colour from geometrical and physical optics, dispersion, scattering, interference and diffraction.

1.1.2.4 Chromophores and Auxochromes

In 1876, Witt [25] proposed that dyes contained two types of groups that are responsible for their colour – chromophores and auxochromes. These original ideas have since been further contributed to and refined.

In order for visible radiation to be absorbed, a compound must contain at least one chromophore [4]. Chromophores, the group of atoms responsible for a dye's colour, are a simple unsaturated group attached to benzene or fused benzene rings [12]. A chromophore is commonly an electron-withdrawing group with the most important chromophores as defined this way being azo (-N=N-), carbonyl (-C=O), methane (-CH=) and nitro ($-NO_2$) groups. The different chromophoric groups are shown below in Figure 3. Double and triple bond groups contain π-bonds beside σ-bonds - but where only the π-electrons are excited. Whereas azo,

cyan, aldehyde, keto and carboxylic acid groups contain non-binding n-electrons [4].

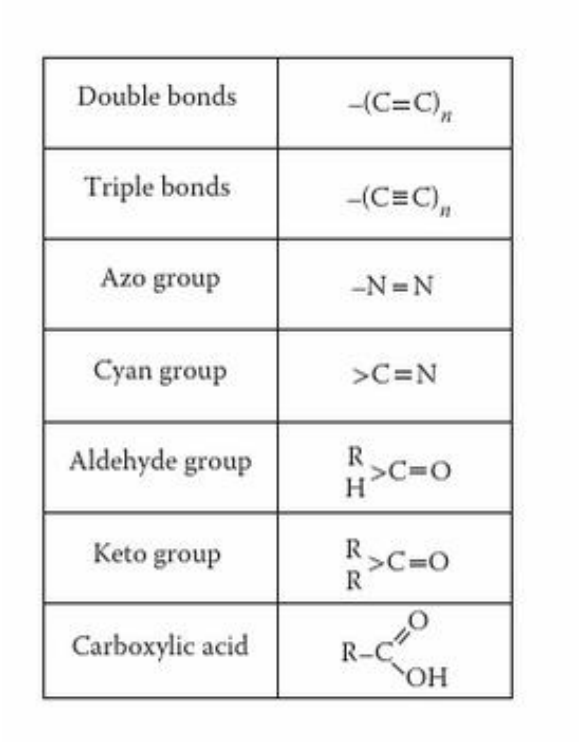

Figure 3: Chromophoric Groups [4]

Auxochromes" are basic, salt forming functional groups such as hydroxyl and amino groups with weakly bonded, easily moveable, electrons that can cause an increase in colour intensity and depth [12]. An auxochrome is an electron-releasing group and they are linked to chromophores through a conjugated system which can cause shifts in wavelengths of the chromophore. Bathochromic shifts in colour (i.e. a shift of the absorption band to a longer wavelength) can be obtained by increasing the electron-withdrawing power of the chromophore, by increasing the electron-releasing power of the auxochromes and by extending the length of the conjugation. Commonly encountered auxochrome groups that normally increase the intensity of colour and shift the absorption to longer wavelengths of light include hydroxyl (-OH) and amino ($-NR_2$) groups [12]. Additionally, some

auxochromic groups such as carboxylic acid (–COOH), sulfonic acid (-SO_3H) and azanide (–NH_2) facilitate bonding to fibres by influencing solubility [4].

Absorption characteristics of a molecule are also influenced by its chemical environment – i.e. for textile fibres the dye's chemical environment is the substrate onto which the dyestuff is bonded [4]. This effect is shown below in Figure 4, where two spectra with different features are displayed. One spectrum is from a purple dye in solution, and the other from the purple dye that has been fixed onto a polyamide 6.6 fibre.

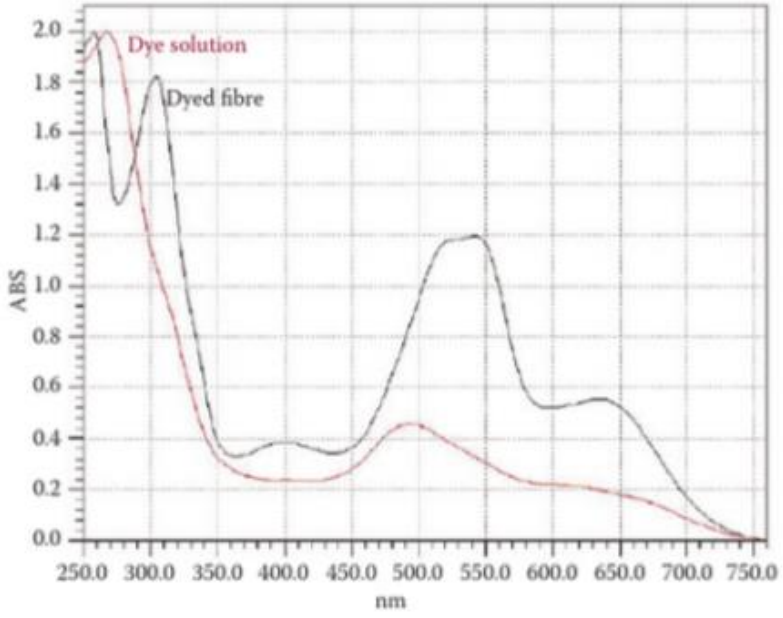

Figure 4: Spectrum of a purple dyestuff applied to polyamide 6.6 compared with the spectrum of a pure dye solution [4]

1.1.3 Dye, Dyeing and Dye Chemistry

1.1.3.1 Pre-dyeing processes

Natural fibres, which may originally be white, off-white, or shades of brown, may be bleached prior to dyeing to remove any natural colour to make subsequent

dyeing easier [3] and/or to brighten and remove imperfections in fibres from different sources [4]. Other processes such as scouring may also be utilised.

Scouring uses alkalis such as sodium hydroxide and sodium carbonate to remove waxes and impurities from fibres such as cotton. Scouring using high quantities of sodium hydroxide (termed mercerising) can also reduce or completely remove the natural twist along the length of the cotton which is present during fibre growth Scouring also increases hydrogen bonding between the molecular chains; increasing the fibre strength by ~20% [4]. Because of increase fibre swelling during this process, the fibrils in both crystalline and non-crystalline regions become more accessible to penetration of moisture – increasing moisture absorbency, comfort and dyeability of the fibre. Increasing the dyeability of a fibre means that a lower quantity of dye is required for a given depth of shade. [4].

1.1.3.2 Dye classes and Direct Dyeing

Dyes are generally classified using their method of application or chemical class [4]. The variation is chemical structure of both natural and synthetic fibres means that some fibre/dye combinations are more common than others; with the method of application of a dye, and the fibre type to which it is applied, is influenced by the relative solubility of the dye in water [4]. Table 2 shows dye classes and their associated textile fibre types.

Table 2: Dye classes and associated Textile Fibre Types [4]

Dye Class	Fibre Type
Acid	Wool, silk, polyamide, protein, polyacrylonitrile, polypropylene
Basic	Polyacrylonitrile, modified acrylic, polyester, polyamide
Direct	Cotton, viscose
Disperse	Polyester, polyacrylonitrile, polyamide, polypropylene, acetate/triacetate
Reactive	Cotton, wool, polyamide
Sulphur	Cotton
Vat	Cotton
Metallized	Wool, polypropylene
Azoic	Cotton, viscose
Ingrain	Cotton

Reactive dyeing is stated as being the most commonly used dye for cotton [4], however, since direct dyeing is utilised later in this research, more information shall be provided on direct dyeing in this section.

Direct dyes are long established for application to cellulosic fibres and derive their name from that they were the first application class to be able to be applied directly to fibres without the need for fixation processes [12]. Direct dyes are almost invariably azo dyes, commonly containing two or more azo groups and are applied directly to cellulosic fibres such as cotton from an aqueous medium containing an electrolyte e.g. sodium chloride [4]. The positively charged sodium

ion is attracted to the negatively charged surface of the fibre (which is caused by the ability of the hydroxyl groups to form intermolecular hydrogen bonds [12]), neutralising the surface and enabling the dye anion to enter the fibre [4]. Direct dyes have some structure similarities to acid dyes used for protein fibres, e.g. the presence of sulfonate ($-SO_3^-$) groups, however the role of this group in the case of direct dyes is to provide water solubility and not dye attraction as in acid dye. This is because an anionic dye may have a reduced affinity for cellulosic fibres due to their negative charge [12].

In general, direct dyes are large molecules that are long, narrow and planar – allowing the dye molecules to align with the long polymeric cellulose fibre molecules and hence maximise the overall effect of the normally relatively weak van der Waals', dipolar and hydrogen-bonding intermolecular forces [12]. Additionally, the introduction of heat during the direct dyeing process swells the fibre as well as increasing the energy of the dye solution components - ultimately increasing the dyeing rate [4].

1.1.3.3 Application of Dye

Natural fibres such as cotton are composed of many different chemical components which are inhomogeneously distributed throughout the fibre matrix - meaning, intra-sample variation in natural fibres is common [4]. Additionally, harvested natural fibres such as cotton can contain immature fibres that dye lighter than mature fibres; causing colour variations due to poor or no dye uptake [4]. Synthetic fibres such as polyester usually have a more homogeneous chemical constitution; meaning in comparison to natural fibres such as cotton, the dye in synthetic fibres is bonded to a relatively constant chemical environment – with the

spectra of dyed synthetic fibres usually showing less intra-sample variation with respect to the wavelength position of the absorption bands [4].

Natural dyes, such as indigo, have been used throughout history; whereas synthetic dyes have gained prominence since World War I [25]. Synthetic dyes are organic compounds and its colour is related to its chemical structure [4]. In 2015, Houck and Siegel reported that ~7000 commercial dyes and pigments are used to colour textiles and ~250 dyes were trademarked with the American Association of Textile Chemists and Colourists [3]. A textile dyer, whose role it is to apply colour to a particular textile fibre, will likely be more interested in the method of application of a dye than its dye class [12].

Dyes are designed to produce materials of a particular colour, intensity, brightness and fastness. To be suitable for a given application, a dye must produce the desired colour. Colour is a pivotal characteristic of fibre evidence that reflects the dyes and pigments applied on the textile fibres [1].

Colour of a sample depends on three factors: the characteristics of the sample itself, the light source used for examination, and the colour response of the eye [26]. Different substances with diverse chemical structures have various reflecting power and absorbency to different wavelengths of light - producing different optical spectra [27].

Fastness refers to the ability of a dye to resist colour change when exposed to conditions such as light, weathering, heat, washing, solvents and chemicals such as acids and alkalis [12]. Therefore dye molecules are designed so they strongly attract to the molecules of the fibre to which they are applied. This can be

achieved in a number of ways including formation of covalent bonds (as in reactive dyeing methods), mechanical entrapment (as in vat, sulfur and azoic dyeing methods) or dye-fibre intermolecular forces such as ionic, dipolar, van der Waals' forces and hydrogen bonding (as in direct dyeing methods) [12]. Direct dyes, in comparison particularly with likes of vat and reactive dyes, provide only moderate wash-fastness – but this can be improved with chemical treatments [12]. Förster *et al.* [28] conducted a photo fading study on cotton dyed with three direct dyes, at different dye depths. They found photo fading occurred after only a few minutes exposure to UV-vis light in each experiment, but this bleaching effect was more pronounced for the lighter shades.

Dyes are utilised for a variety of products, including; clothing of all types, curtains, upholstery and carpets. Dyes are almost exclusively applied to textile materials from an aqueous medium and are therefore required to readily dissolve in water [12]. Examples of dye methods that are readily dissolvable in water include: acid dyes, mordant dyes, premetallised dyes, direct dyes, cationic dyes and reactive dyes. Vat and sulfur dyes, which are commonly utilised with cellulosic fibres, are completely insoluble in water but are converted into a water soluble form by chemical reduction prior to application to a fibre. Disperse dyes and pigments are insoluble, unless applied at high temperatures, and are applied via a dispersion process where they remain solid particles and are held in place mechanically e.g. in a matrix of a solid polymer [12].

In textile dye houses, where textile materials are dyed in batches of varying size, the contents of the dye baths used to produce a colour shade are not constant but are varied by using different dyes [4]. This is a process known as "topping up" [29]. No single dye is used to create a particular colour and even simple dyes may be

put through up to 10 processing steps to achieve their final dye form, shade and strength [3]. Ultimately, millions of shades of colour are possible in textiles [30].

1.2 Textile fibre evidence in forensic science - Challenges Facing the Forensic Science Community

The situation of fibre evidence in parts of Europe and the UK has been discussed at the 2017 European Textile and Hair Group (ETHG) where the value of fibre evidence was determined to be “undisputed among forensic scientists, but not always recognised by police and legal representative”[13]. Since the publication of the United States National Research Council of the US National Academy of Sciences report in 2009, also known as the “NAS Report”, an emphasis has been placed on the fundamental science that underlies forensic investigation; resulting in the need for a comprehensive assessment of the capabilities, limitations and application of various analytical techniques [31]. In short, there is a need to improve the reliability of forensic science. This is a requirement across all evidence types before the confidence in any result should be communicated. Furthermore, The House of Lords Science and Technology Select Committee [32] recently stated that judges were keen to see more research on evaluating the significance of a “*match*”, once one has been discovered, and noted there is little research into analysis such as machine learning in a forensic context to date.

Variability exists across the forensic science disciplines with regard to techniques, methodologies, reliability, numbers and types of potential errors, general acceptance, and amount of published material in that field [31]. Those forensic science disciplines that are laboratory based and viewed as having methods that are more robust, transparent and rigorous such as DNA analysis, in the eyes of the US National Research Council, are more reliable than those that are based on expert opinion and experience alone such as fingerprints, handwriting analysis, and specimens such as hair and fibres [31]. The 2009 US National Research

Council report states it is their belief that "*with the exception of nuclear DNA analysis no forensic method has been rigorously shown to have the capacity to consistently, and with a high degree of certainty, demonstrate a connection between evidence and a specific individual or source*" [31]. The report noted there are variations within the "subjective" disciplines (i.e. those relying on opinion based interpretation) such as textile fibre evidence, whereby the interpretation of the evidence can rely on the past experiences of the analyst. It is therefore possible that these variations in interpretation, although uncommon, could result in disagreement between experts [33–36]; leading to different evaluations and interpretations being formed from the same evidence and information.

In discussion around DNA evidence in the National Research Council report, the term "match" (or terms including "match") are mentioned – with this often being in the context of determining the likelihood of a "match" having originated from person X rather than another unrelated person. This determination of a match between profiles is a key element to DNA evidence process; albeit one that does not solve the case or answer all of the questions. After all – a DNA "match", like other evidence types, can be misinterpreted in the absence of context. For example, finding DNA from semen in a stranger rape case compared to in a consent defence argument has a profound effect on the evaluation of the prosecution and defence hypotheses [37]. This means that some form of logical human evaluation of competing hypotheses is still required throughout this perceived robust and accepted process adopted by DNA evidence. In this research, a "match" means that two or more fibres are indistinguishable with regards to the information provided by microspectrophotometry (MSP).

1.3 Forensic Examination of Textile Fibres

The forensic examination of textile fibres is routinely carried out in forensic science laboratories across the world. Fibre evidence has applications in investigating various crimes, including serious crimes such as sexual offences and homicide [38]. Fibre evidence can help to provide information to answer questions of “when” and “how” in a given case, by providing links between individuals, individual(s) and scene(s), or two (or more) scenes [4]. However, given the rise in utility of DNA analysis, trace evidence, such as fibres, have seen a decrease in their utilisation in criminal investigations; potentially stemming from a misconception of their potential value to an investigation [24,39]. It is believed that these misconceptions may come from poor casework assessment and reporting - as well as perceptions of high labour intensity, poor evidential value and poor cost effectiveness [38].

A traditional pathway of fibre examination to determine if two textile fibres are indistinguishable and therefore could have come from the same source (as garments are not unique it is not possible to say with complete certainty that fibres have originated from a specific garment) begins with visual examination and low powered microscopy - observing broad colour of the sample as well as some easily observed morphological features such as cross sectional shape. Following this, other forms of microscopy including polarised light microscopy (PLM) and high powered comparison microscopy can be performed [40,41]. PLM is used to identify the generic class of a fibre, and high power comparison microscopy allowing for the direct comparison of morphology, diameter and colour between samples utilising a variety of light sources. PLM however cannot reliably differentiate between different members of the same generic class, e.g. nylon 6 and nylon 6.6.

If, following these visual comparisons, the analyst deems two samples to be indistinguishable then further examination using microspectrophotometry (MSP) would be performed.

1.4 Microspectrophotometry (MSP)

1.4.1 Introduction

A dye is an organic chemical that is able to absorb and reflect certain wavelengths of visible light [3]. Historically, there have been multiple different methods utilised for colour analysis in textile fibres including; visual examination [3], spectroscopic methods [42–44], infrared (IR) spectroscopy [45,46], Raman spectroscopy [47,48]) and chromatographic methods (e.g. thin layer chromatography (TLC) [49,50] and high performance liquid chromatography (HPLC) [51,52]).

Microspectrophotometry (MSP) belongs to the wide range of “spectroscopic methods” and is a combination of microscopy and spectrometry whereby electromagnetic radiation of different wavelengths is used to examine different aspects of a dye’s behaviour based on molecular structure [4]. Spectroscopic methods are unlike chromatographic methods that require the extraction of dye before analysis [1]. Dye extraction from fibres can be difficult due to their small size, and textile dyers typically want to ensure that dye stays within the fibre under most conditions. Additionally, dye extraction is a destructive method, making the fibre useless for further colour analysis [3] and so must be applied with caution and almost certainly when all other analysis options have been exhausted.

A diagram of a fibre mounted for analysis, an MSP setup, and the resulting spectrum is shown below in Figure 5.

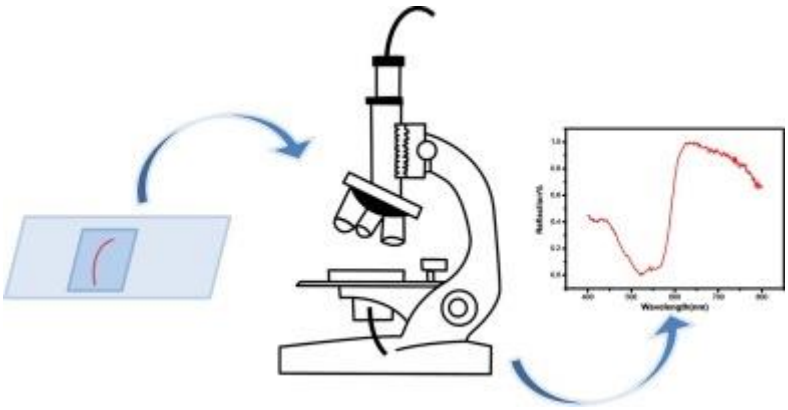

Figure 5: A diagram of the mounted fibre, MSP and the resulting spectrum [1]

The influence of the environment of the dye (i.e. the fibre itself in the case of textile fibres) on the shape of the spectrum has some practical consequences [4] and ultimately results in difference spectra being produced between the dye in solution and the dyestuff in the fibre (seen previously in Figure 4).

The microscope component of the MSP system is used to place the object in the stage plane and allow reproducible focussing of the radiation onto the sample before the spectrophotometer compares the amount of light passing through air with the amount of light transmitted through the fibre sample [3,4]. Fibres tend to be long, linear objects (with typical lengths at least 100 times greater than their diameter) meaning a long, narrow rectangular shape is the most suitable measurement slit which must be centred within the fibre [4]. This process is again aided by the microscope component of the MSP.

1.4.2 The application of MSP to the analysis of textile fibre dyes.

Fibres encountered as forensic evidence, both synthetic and natural in origin, are originally opaque; but single dyes or a mixture of dyes are added to them to make them commercially useful [4]. The human eye-brain system, although rapid and

extremely useful as a searching and screening tool can be prone to difference from factors such as fatigue, genetics, age, sensitivity and eye adaption – meaning that colour impression from the same sample may vary not only from person to person, but also the same person at different times [3,4]. Even when two colours are determined to visually match, one cannot be sure if both samples are actually dyed with identical dyes. Objects which appear the same visually under a given lighting condition, but are dyed with different dyes or mixtures of dyes are called metameric [3,4].

The European Textile and Hair Group (ETHG) and the Scientific Working Group for Materials (SWGMAT) Fibres Section in the USA tested a variety of MSP systems, utilising 42 laboratories in 21 countries, and found that MSP systems produced “reliable and comparable” dye spectra [4]. Using MSP, the colour of the sample is collected - allowing for the objective measurement of absorption of electromagnetic radiation [40]. This information is then output in the form of a spectrum which can be compared by the analyst to other collected spectra as a tool during their evaluation as to whether or not two of more groups of fibres originated from a putative source.

MSP is commonly utilised in the visible range (~400 nm – 800 nm) [53,54] and can include the UV range (~200 – 400 nm) for additional information which may aid with discrimination [55]; depending on the equipment available and the fibre type being examined. Some fibre types, such as polyester, will absorb UV wavelengths and therefore no additional information would be provided using UV range analysis [16,56].

MSP is commonly used in forensic science as it is a quick and non-destructive method for examination of fibres [1,29,57–73]. MSP enables the measurement of colour from small samples such as textile fibres [1] but also has application to other coloured trace evidences such as inks, paints, hair , soil and blood [57,73–83]. Whatever wavelengths are utilised, the obtained MSP spectra provide a controlled, verified and objective method which helps eliminate the subjectivity associated with human description of colour and separate metameric samples [3,4,16]. As such, MSP is an industry standard technique used for the comparison of colour in the forensic examination of textile fibres when comparing fibres recovered from a crime relevant substrate with a putative source [40].

1.4.3 Underlying principles of MSP

1.4.3.1 MSP and the produced spectra

One of the most important early contributions to the science of colour was by Witt in 1876 [25] who proposed that dyes contain two types of groups responsible for their colour: chromophores and auxochromes. These concepts are described in more details in previous sections. Also as previously discussed, there are two ways that two or more colours can be mixed to create new colours: additive and subtractive colour mixing. Subtractive mixing is involved when colours are observed as a result of reflection from or transmission through an object after it interacts with incident white light. Subtractive colour mixing is the process involved when dyes and pigments are mixed and are therefore of most relevance to MSP [12].

Measurement of light by MSP involves the interaction between electromagnetic radiation and dyes in the fibre. Absorption of light is calculated based on two

values; radiation flux in the system without the object (I_o) and radiation flux with the object (I) [4]. The equation for absorbance (A) is shown below in Equation 2.

Equation 2: Calculating Absorbance [4]

$$A = log\ \frac{I_o}{I}$$

An electron in the dye molecule becomes excited if the frequency (and therefore wavelength) of incident electromagnetic radiation matches or closely corresponds to the difference in energy between two electronic states. This leads to resonance excitation, change in electron density distribution followed by an electronic transition from the highest occupied molecular orbital to the lowest unoccupied molecular orbital [4]. In the UV-vis region of the electromagnetic spectrum, the absorption of radiation is caused by the excitation of valency electrons of which there are three types: σ-electrons of the molecular frame, π-electrons of the double bonds and triple bonds, and the pairs of non-binding n-electrons [4]. Because UV-vis MSP is typically utilised wavelengths above 240 nm, sufficient energy is not present and therefore excitation of the σ-electrons does not occur and instead MSP relies on the excitation of π- and n- electrons which require less energy. Conjugation of double and triple bonds leads to easier excitation and therefore can occur using wavelengths in the visible range – giving the substance its coloured appearance [4].

The spectrum produced is not a line spectra, due to vibrational and rotational transitions occurring in addition to the excitation of electrons – consequently making the absorption bands typically broad [4,12]. The spectral information of absorbance caused by a dyed fibre using MSP is a combination of; intensities and wavelength positions of absorption maxima and minima, shapes of peaks troughs

and shoulders, and inclines and inflexions of sections of the spectra [4]. Examples of these features are shown below in Figure 6.

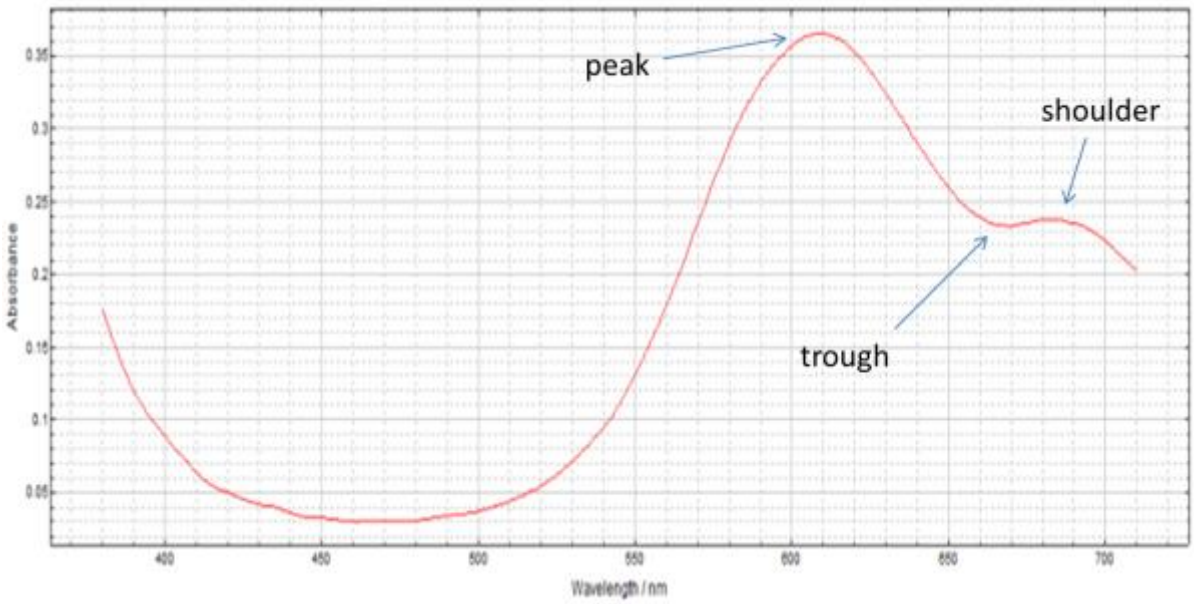

Figure 6: A visible range MSP spectrum obtained from a blue cotton fibre demonstrating some common features; shoulders, peaks and troughs.

Initial observations of spectrum detail outlined above can be quickly made by the human eye-brain system [3] before the use of other methods such as overlaying spectra. Some dye processes can also give rise to very characteristic appearances: such as the appearance of “tiger tails” in acrylic fibres, or the “over-dyeing” of fibres where the original colour(s) are still visible. These features make a fibre more unique, and therefore increase their evidential value [4].

Ultimately, the analysis of MSP spectra involves analyst carefully comparing the shape of the spectra obtained between two or more samples across all the wavelengths used for examination. The dye(s) used with the sample, as well as the dye concentration, result in varying absorbance values being obtained at all examined wavelengths; influencing the shape of the spectrum produced. If for

some or all regions of the spectra, samples are considered to differ, when taking into account sample variation, then the MSP spectra from the groups of fibres are deemed to be distinguishable and therefore the samples have originated from different sources. On the other hand if spectra of the samples are considered to be indistinguishable across all the wavelengths, when taking into account sample variation, then the samples *could* have originated from the same source.

1.4.3.2 Colour of a sample

The colour of a sample depends on three factors: the characteristics of the sample itself, the light source applied, and the colour response of the detector [84]. Different substances have different reflecting power and absorbency to different wavelengths of light – ultimately producing different optical spectra [26,27]. Colour can be described in a number of ways, with one such way being in terms of shade (hue), intensity (strength) and brightness [12].

Shade is determined by the wavelengths of absorbed light and can be investigated using the λ_{max} values obtained from UV-vis MSP. In terms of a UV-vis spectra, the brightness of a colour is characterised by the shape of the absorption band; with dyes that have narrow bands being bright, and dyes that have broad bands being dull [12]. Intensity can be measured using the molar extinction coefficient (ε) at the λ_{max} value. This can be obtained from the UV-vis absorption spectrum of the low concentration dye in solution using the Beer-Lambert Law (Equation 3); where A = absorbance at a particular wavelength, c is the concentration of the dye and l is the path length [12]. However, since the strength of colour is related to the area under the absorption band, this relationship with ε should be treated as qualitative and dependant on the shape of the absorption curve.

Equation 3: Beer-Lambert Law [12]

$$A = \varepsilon c l$$

An increase in intensity (i.e. a higher dye concentration) leads to an increase in the absorbance value obtained, and also therefore an increase in the area under the curve. The dye concentration, and therefore intensity, can be determined during the manufacturing process for a textile fibre – however from a practical standpoint it is important to appreciate limitations of the MSP in terms of recording absorbance units (AU). Values higher than ~1.4 AU recorded using MSP tend to produce plateaued peaks [65] – making λ_{max} determination much more problematic [53].

Similarly, when fibres are very lightly dyed, the colour differentiation according to MSP may be difficult as noisy spectra are produced [53]. Uncoloured fibres are also difficult to analysis using MSP as they lack the dyed molecules and necessary colour components for successful analysis. Additionally, because of how common white cotton is, it is usually evidentially worthless [4]. However, uncoloured fibres can fluoresce when utilising UV range MSP - owing to the presence of fluorescent brighteners [1,12].

1.4.3.3 Intra-sample variation

The colour of natural fibres, such as cotton, often display more intra-sample variation than synthetic fibres; and the observed colour may vary along the length of a natural fibre due to differential dye uptake [3,29,58,85]. To combat this, greater numbers of natural fibres are typically measured compared to synthetic fibres to ensure suitable representation of spectral variation in the sample [4]. It

has been argued that variations in amount of wear, bleaching and laundering may cause artefacts which increase the colour variation with a textile fibre of source – meaning that is has not been possible to completely standardise the number of known fibre samples selected for a given analysis [4,44]; but generally ten natural fibres or five synthetic fibres as a minimum would normally be measured [4,41].

1.4.4 Previous applications of Microspectrophotometry (MSP)

1.4.4.1 History

Microspectrophotometry (MSP) was first pioneered in 1868 by Sorby and Browning who combined a microscope and direct vision spectroscope and initially applied this instrument to the study of colours of natural pigments in biological material; with the first application of MSP to forensic science was the microspectral study of blood [1]. The application of MSP to the forensic examination of fibres was first described by Amsler in 1959 [86]. Then, in 1986, Laing *et al.* [87] reported the use of MSP to discriminate visually identical fibres based on their visible absorption spectra.

1.4.4.2 Discriminating power (DP)

The discriminating power (DP) of MSP for textile fibres, where the proportion of samples that can correctly be distinguished is calculated, was first evaluated by Macrae *et al.* in 1979 [88] who examined blue and red wool fibres and found the DP of red wool to be 0.94 and blue wool to be 0.99. More recently, the main developments of MSP from a forensic science aspect include the ability to examine other analytes and an increased degree of discrimination [38] i.e. being able to provide a higher discriminating power [89]. Studies utilising MSP for textile fibre analysis, including DP where reported, are highlighted below in Table 3.

Table 3: Summary of the discriminating power of MSP in various studies

Author(s)	Year	Fibre(s) Examined by MSP	Discriminating Power (if reported)
Grieve *et al* .[90]	1988	Blue cotton Red cotton Black cotton	Not reported
Grieve *et al.* [91]	2001	Black cotton	Up to 0.93 (reactive dyes)
Grieve *et al.* [92]	2003	Orange cotton Green cotton	0.93 0.998
Grieve *et al.* [22]	2005	Blue polyester	0.991
Cassista and Peters [93]	1997	Red cotton Green cotton blue cotton	Not reported
Robson [94]	1997	Red cotton	Not reported
Biermann [62]	2007	Blue cotton Red cotton	0.9996 0.9995
Palmer *et al.* [59]	2009	Blue cotton	Up to 0.96

In the past years, a lot of research has been conducted on cotton fibres as this is the most commonly encountered fibre type. Similarly for synthetic fibres, polyester has seen increased reporting as polyester has seen an “extensive increase in production” [16]. Table 3 demonstrates that MSP is a highly discriminating technique. In 2009, Walbridge-Jones discussed the strengths and limitations of MSP for textile colour measurement [95] but since then, to the authors knowledge, no review articles had been published that summarised recent developments in

the colour analysis of textile fibres by MSP until Hu *et al.* in 2020 [1]. Additionally, no case reports were published between 2016 and 2019 according to Lepot, Lunstroot and De Wael [13].

Discrimination of fibres is discussed further in section 1.6.

1.4.4.3 MSP alongside other techniques

Eng *et al.* [96] previously utilised UV–vis MSP to analyse metameric samples which were created using different colouring agents or different relative concentrations of the colouring agents. As well as MSP, microscopy and thin layer chromatography (TLC) have also been employed for comparison of the dyes to examine the batch variation [97]. MSP and TLC are generally viewed as complimentary techniques, with MSP being able to discriminate fibres that cannot be discriminated by TLC alone [1,53].

Massonnet *et al.* [66] employed Raman spectroscopy and MSP to analysis a binary reactive dye mixture on cotton fibres. The detection limit of Raman was found to depend both on the chemical composition of the dye itself and on the analytical conditions, particularly the laser wavelength. For dye mixtures, Raman spectroscopy was more sensitive compared to MSP. Although suitable for the binary dye mixture, Raman is limited when applied to more complex mixed dyes. Also, as noted by Lepot *et al.* [98], some interference from mounting resin and glass slides can be observed when examining fibres mounted for MSP analysis directly using Raman spectroscopy.

1.5 Potential issues of the current approach

Currently, the determination of whether or not two or more textile fibres are indistinguishable or distinguishable relies heavily on expert experience and opinion. Although some aspects are objective such as the spectra produced using MSP, the current prerequisite visual comparison stages of microscopy, colour interpretation as well as the subsequent MSP spectra comparison are largely subjective and based on opinion. Although uncommon, this can lead to disagreements between fibre examiners as previously stated.

A 2016 report by Palenik *et al.* [65] stated "*there is currently no practical source of information to assist examiners in the interpretation of [MSP] spectra*". As the report originated in the United States, the authors likely based this statement on source level comparisons i.e. comparing two groups of fibres to determine if they are indistinguishable and therefore may have originated from a putative source. In their report, they describe their process of interpretation between spectra collected from two different purple fibres, with these spectra being shown in Figure 7.

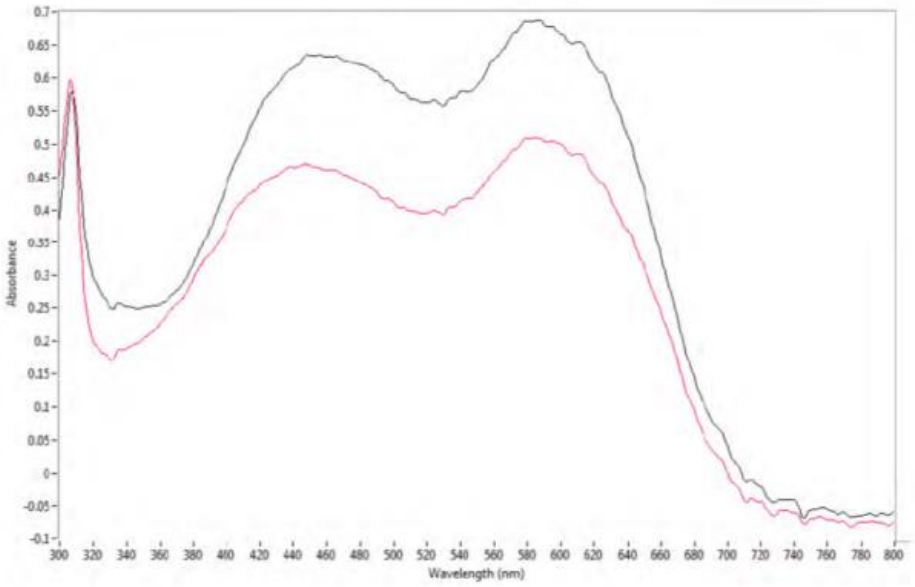

Figure 7: The spectra from the two purple fibres as discussed by Palenik *et al.* [65]

The spectra in Figure 7 were said to be very similar - however very slight differences in colour observed under a comparison microscope (as well other independent facts from the case) "*proved definitively*" that these fibres were from different sources [65]. The authors note that such determination was made on the basis of the experience built upon looking at "*hundreds, if not thousands, of spectral comparisons*" – demonstrating how opinion based interpretation can rely on previous experience of the examiner.

An aim of this research is to consider situations such as the one the statements above exemplify (i.e. area of potential disagreement the subjective and opinion based interpretation of textile fibre evidence). Although some areas of the examination of textile fibres, such as the spectra generated when using MSP are objective and cannot be influenced by the analyst, the comparison of MSP spectra visually is much more subjective and can be influenced by the analyst's experiences.

1.6 Discrimination of fibres

Since the 1990's there has been a movement towards arguably a more balanced, logical and robust method of interpretation of reporting casework examinations utilising a Bayesian approach [99–104]. In 2001, Grieve and Wiggins [104] cited a number of issues, specifically aimed at improving the effectiveness of forensic fibre examinations - including the implementation of a streamlined analytical process, utilising the more discriminating techniques such as MSP prior to the more traditional comparison microscopy step - as well as the need for data vital to evidence evaluation and interpretation.

It has been argued that, even recently, some fibre examinations continue to follow a scheme of work which predates technological advancements by spending large amounts of time and resources on less discriminating or unsuitable methodologies [38,105]. This is obviously detrimental to forensic science given the ever present drive within to decrease turnaround time and increase cost efficiency while still providing a robust interpretation of fibre examinations. These points were highlighted in 2001 by Grieve and Wiggins [104] and continue to be an ongoing struggle as evidenced by the production of the National Research Council Report [31] as well as the various reports by the Forensic Science Regulator - including the latest report at the time of writing [39].

It has therefore been suggested, that the thought and examination processes should be revisited and updated to reflect technological advancement in order to potentially improve the efficiency of the textile fibre examination process and combat some of the negative perceptions as mentioned previously, such as poor efficiency and cost effectiveness [38].

An argument for an updated approach to fibre evidence examination was exemplified by Palmer and Booth [105], when they considered the approach when examining blue cotton fibres. Blue cotton represents one of the most commonly encountered fibre colour/type combinations in both population studies and casework [7–9,15,22,106]. However, this commonality has resulted in a misinterpretation of the potential value of this evidence type – with the flawed logic being that because blue fibres are so frequently encountered in the general population, they have less significance to an investigation.

This likely stems from a misinterpretation of the available population studies which obtain frequency data at a very generic, conservative level; but only define a broad fibre type/colour group to see a larger picture without an impracticable large number of subgroups [38]. There is therefore a risk that this misinterpretation could occur with members of the criminal justice system; resulting in the evidential value of some evidence types being largely and unjustifiably understated.

In the views of Grieve [107] , Palmer [38] and Booth [105] between 2000 and 2016, the discrimination afforded by the range of MSP equipment, particularly when used in combination with other techniques or extended to include the UV range, did not seem to have been factored into the interpretative process.

Studies by Grieve *et al.* [91,108] investigated the discrimination of the most commonly encountered cotton blocks of colour; namely blue, red and black. These studies demonstrated that microscopy alone, which would traditionally be a commonly used examination technique early in the investigative process, offered very little discrimination. This discrimination was increased considerably when visible range MSP was performed.

Following this, research was performed which included the use of the UV range MSP examination. Biermann [62] showed using the discrimination power of UV-vis range MSP, in combination with microscopy, allowed for red and blue cotton fibres to be readily distinguished. Palmer *et al.* [59] further investigated blue (non-denim) cotton fibres using MSP alone. The authors were able to subdivide 100 fibre samples into two subgroups (mid blue and dark blue – with light blue being excluded due to being too pale and producing poor quality spectra), before being able to identify further divisions within these two broad subgroups. Their reported discrimination power of 0.96 for both mid blue and dark blue garments were determined to be similar to previously reported studies which also incorporated light and fluorescence microscopy – giving further weight to arguments of a more streamlined approach prioritising MSP analysis.

The results of these studies informed the proposal of an alternative examination process - prioritising MSP over visual examinations such as microscopy as a “first test” in the fibre comparison sequence; particularly when considering commonly occurring fibre/colour combinations such as blue cotton. Blue cotton was selected as a suitable combination for this alternative approach as non-denim blue cottons exhibited a very low discriminating power when examined using microscopy alone.

Going even further, the research presented in this thesis considers the use multivariate analysis to aid with the interpretation process, allowing for an objective and probabilistic approach to determine if two groups of fibres are indistinguishable or distinguishable based on the information provided in the spectra as opposed to relying solely on opinion based interpretation.

1.7 Multivariate Analysis

Multivariate analysis is becoming more commonly used in the forensic community (a) for discriminating data and (b) for providing an objective comparison of data [67,71,109,110]. These techniques are very convenient to use for highlighting small or even tiny variations in spectral data for instance [13]. Multivariate analysis is performed every time a relationship is attempted to be established between multivariate data (i.e. data with multiple responses or variables). In this research specifically, multivariate analysis is used to train a classification algorithm in a case-by-case approach - training it on case-related data and not on a previous existing and general database approach. The database approach is perfectly acceptable when suitably large and stable databases are available – such as those used for DNA interpretation. However, when database size is not suitable, or the data within is not stable (e.g. the footwear database used during the RvT case [111]) then this probabilistic based approach of this research would be stronger than relying on frequency data only.

1.8 Previous uses of multivariate analysis in forensic science

Multivariate analysis uses measurements from multiple variables (such as absorbance taken over a wavelength range in the case of this research) to identify patterns and groupings from large, complex, datasets more reliably than is possible by visual examination of datasets alone [67]. The use of MVA has been investigated previously using various evidence types including drugs [112,113], inks [77,79,80,114,115] and paints [81,82,116]. Many of these studies have involved the use of techniques such as hierarchical clustering analysis (HCA), principal component analysis (PCA) and linear discriminant analysis (LDA); which are generally the most common approaches observed by the author during literature searches.

1.9 Recognising subjectivity

Subjectivity, as is considered for the purposes of this research, relates to evaluation of evidence that is influenced by an examiner's perception or their previous knowledge/experience - for example, the visual comparison of MSP spectra shape/features, the colour observed using microscopy and if differing dye variations are common for a questioned fibre. The National Research Council report comments that the large amount of research into DNA has allowed for development of analysis has become "*less subjective*" [31] and therefore more reliable. Other areas of forensic science have not been as fortunate with respect to the amount of research conducted and the amount of current, and stable, data that is available to build an accepted accurate and robust database - with textile fibres being no exception. With regards to stability, as stated by Palmer [38], *"The fundamental difference between DNA evidence and fibre evidence is that; data relating to the prevalence of the former is fixed in time, whilst the latter is not"* due to previously discussed factors such as changes in climate and fashion. However, there is a now a desire for the development of less subjective analysis and interpretation.

In the author's view, in order for a proposed technique to be objective there must be three main criteria: a probabilistic approach; minimal user input required; and a set of inputs that yield reliable recommendations across a variety of different samples e.g. different fibre types, different colours.

Given the limitation where only a select number of fibres may be available to produce a sample set for analysis, as well as the subjective elements of fibre examination that may lead to disagreements between examiners, it is felt that the development of a more objective multivariate analysis/machine learning method

which can provide input as to whether MSP spectra from two groups of fibres are indistinguishable or distinguishable would be beneficial. This would provide information to contribute towards “sub-source” level, which is vital for working at both source and activity levels - and would be a complimentary benefit to the current fibre examination and interpretation process.

The requirements for a proposed classification system, if the proposed system were to be suitable for potential applicable to forensic casework, are outlined and discussed in detail in the following chapters.

1.10 Aims and Objectives

The aim of this research is to determine whether multivariate analysis, specifically principal component analysis and linear discriminant analysis, can be successfully applied to textile fibre evidence to objectively classify if two groups of MSP spectra from fibres are indistinguishable or distinguishable.

In order to achieve this, the following objectives will be explored and discussed in the subsequent chapters:

- Outline the requirements for an “ideal” classification system.
- Determine the optimal settings to allow for high accuracy when using fibres from clearly (visually and spectrally) distinguishable sources, as well as fibres from the same source and should therefore be indistinguishable.
- Determine if these optimal settings can be successfully applied to the most common blocks of colour encountered in forensic science i.e. where fibres will be less obviously visually and spectrally distinguishable.
- Determine the limits of sensitivity of the proposed methods when examining fibres of the same material, with differing proportions of dye present in each bulk sample.
- Propose a methodology when working with single fibres – one of the most challenging situations for a fibre examiner.

2. Materials and Methods

2.1 Sampling and mounting fibres

2.1.1 Fibre Shade Cards

Two different fabric shade cards, cards with fibre samples used for selecting colour(s), were used during this research - one comprising acrylic fibres and the other comprising cotton fibres. These two fibre types were selected based on availability to the researcher and that they represent commonly encountered synthetic and natural fibre types respectively in studies published across the world between 1997 and 2015 [6–10,15,21,62,91,106].

Acrylic fibres were taken from a J&P Coats Plastic Canvas Yarn shade card (Coats Patons Crafts). To determine if any of the 33 samples on the shade card were not suitable for further analysis due to the quality of their microspectrophotometry (MSP) spectra, 10 fibres were analysed from all 33 samples were analysed using visible range (380-710 nm) MSP. From the 33 available acrylic samples, 10 were excluded due to insufficient spectral detail arising from the fibres being too pale. Fibres with insufficient spectral detail produce spectra which are noisy and/or featureless and were therefore of little use for discrimination [3,53,59,117]. The 10 excluded samples were: “white”, “natural”, “maize”, “sea coral”, “lily pink”, “cameo rose”, “lavender”, “sky blue”, “pale green” and “pale grey”. The remaining 23 samples were used for further examination and experiments. The acrylic shade card, including an illustration of the excluded samples, is shown in Figure 8.

Figure 8: Scanned image of the acrylic shade card from which the 23 difference colour samples were obtained

Cotton fibres were taken from an Anchor Embroidery Threads 30 shade card (Coats Mez). In an attempt to ensure comparable samples between the cotton and acrylic fibres, cotton samples that were most visually (but not necessarily spectrally) similar by eye to the previous 23 acrylic samples were selected for further examination.

2.1.2 Mounting fibres for visible range (380-710 nm) microspectrophotometry

2.1.2.1 Phytohistol mountant

Phytohistol was selected as the mounting medium for visible range (380 – 710 nm) MSP as it is colourless, quick drying and stable. Additionally, it does not have the same health and safety concerns as some xylene based mountants which have been researched previously [118,119] making it safer and more likely to be used in current forensic investigations. Phytohistol mountant was made by mixing 100 mg potassium benzoate (Sigma Aldrich), 70 mg citric acid (Sigma Aldrich) and 40 mL of distilled water while gently heating until fully dissolved. The mixture was then removed from the heat and 120 mL sugar syrup added and stirred into the hot mixture [120]. The phytohistol was then stored in brown glass bottles in a cool, dark place until required.

2.1.2.2 Fibre Scraping and mounting

To ensure cross contamination was reduced, the area to be sampled from the shade card or fabric sample (as well as the surrounding area of the shade card/fabric to be sampled) was taped using Sellotape™ to remove any extraneous fibres which may have been deposited during storage or previous scraping of nearby samples. These fibres extraneous to the intended source, if analysed by MSP, would constitute contamination and may result in the spectra being obtained for each sample not being fully representative. This may in turn have a negative impact on the subsequent interpretation by the proposed multivariate analysis (MVA) system.

After taping to remove extraneous fibres, fibres were scraped from the required area of a shade card or fabric using a clean scalpel (Swann Morton Limited). Scraping was used for obtaining fibres from the samples as opposed to tape lifting to allow for a more efficient process by removing the need for searching for, removing and mounting the fibres after tape lifting. Tape lifting is more commonly used for recovering transferred fibres rather than creating control samples where scraping and plucking are utilised more commonly – however when examining the fibre dye using MSP, any areas of observed damage on the fibre was avoided [65,121]. A small drop of phytohistol was placed onto a glass microscope and the scraped fibres transferred to the phytohistol using tweezers (Taab Laboratory Equipment Ltd). A glass coverslip (Scientific Laboratory Supplies Ltd) was then placed over the fibres and the slide kept in a dark cupboard for at least 24 hours before being examined to allow the mountant to set and minimise any photo bleaching of the fibres occurred from exposure to sunlight [28,122]. Between samples, the scalpel and tweezers were cleaned using Sticky Stuff Remover™ (Orange-Sol Companies) to remove any residual fibres and reduce cross contamination.

2.1.3 Mounting Fibres for UV-vis range (280-710 nm) microspectrophotometry

The above process of taping prior to scraping was repeated. Fibres were scraped from the required area of a shade card or fabric using a clean scalpel. A small drop of *glycerol* (BDH) was placed onto a *quartz* microscope slide (Agar Scientific) and the scraped fibres placed into the glycerol using tweezers. Quartz slides and glycerol were used for UV-vis range MSP as they do not absorb in the UV range [1] (unlike glass and phytohistol) and glycerol has no associated health and safety

concerns unlike some other suitable UV mountants [119]. A quartz coverslip (Agar Scientific) was then placed over the fibres and the slide kept in a dark cupboard until being examined. As before, the scalpel and tweezers were cleaned using Sticky Stuff Remover™.

Note: As it was not feasible to take a measurement from the exact same fibre, rather than remounting the individual fibres, new samples were made from the shade cards. Fibres for UV-vis range MSP were re-sampled from the shade card or fabric as opposed to being remounted from the original glass slides. This was due to a limited number of quartz slides being available with the available research funds in comparison to the number of glass slides - meaning the fibres mounted on the glass slides could be kept indefinitely whereas quartz slides needed to be reused multiple times and did not allow for indefinite storage of fibres for UV-vis range analysis.

2.2 Microspectrophotometry (MSP)

A J & M TIDAS MSP 800™ microspectrophotometer (J&M Analytik) was used alongside ONYX software (Faraday Scientific Ltd) for spectra acquisition. The microspectrophotometer was turned on and allowed to warm up and stabilise for at least 30 minutes before use, as per the manufacturer's recommendations. All spectra were acquired using a 40x objective lens and a measurement window of 4.65 x 48.825 µm as set during the service and calibration of the MSP by the manufacturer. These settings could not be compared to other published studies involving MVA and MSP as they were not noted in other publications.

Dyed natural fibres, such as cotton, often exhibit greater intra-sample variation compared to dyed synthetic fibres, such as acrylic, due to differences in dye uptake resulting from lesser uniformity along the length of natural fibres [3,58,59,68]. To minimise intra-fibre variation, and any anomalies caused by a single point in the fibre, three measurements were taken at intervals along the length of each fibre - with the three measurements used to produce an average spectrum for each fibre. This was repeated for each of the (up to 40) fibres being investigated for each sample.

As noted previously, samples were selected based on how visually similar they were to the eye and not necessarily by how similar they were spectrally when spectra were obtained from MSP (i.e. were metameric samples [69,85,95,96]). Examples of the differences observed in the spectra of visually similar red and blue sources (i.e. visually similar red acrylic vs. visually similar red cotton) are shown in Figure 9 and Figure 10 respectively. In each figure, each group of 10 fibres are from the same source – demonstrating repeatability. The differences

between the spectra produced, even when fibres appeared similar to the naked eye, demonstrate that MSP is able to differentiate metameric samples.

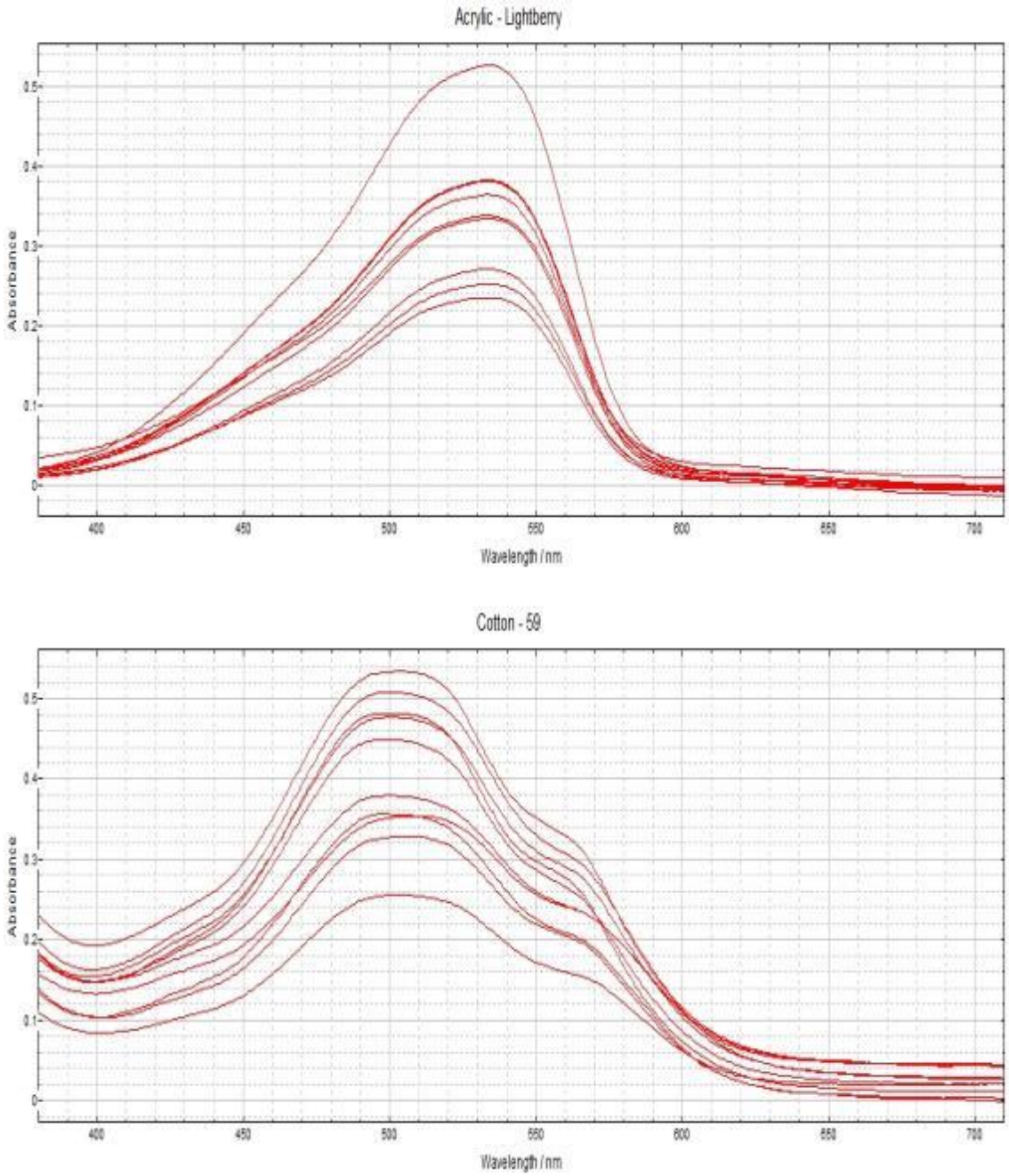

Figure 9: Demonstrating the spectral differences between two visually similar red samples; acrylic "Light berry" (top) and cotton "59" (bottom). Each 10 fibres are from the same source – showing intra sample variability.

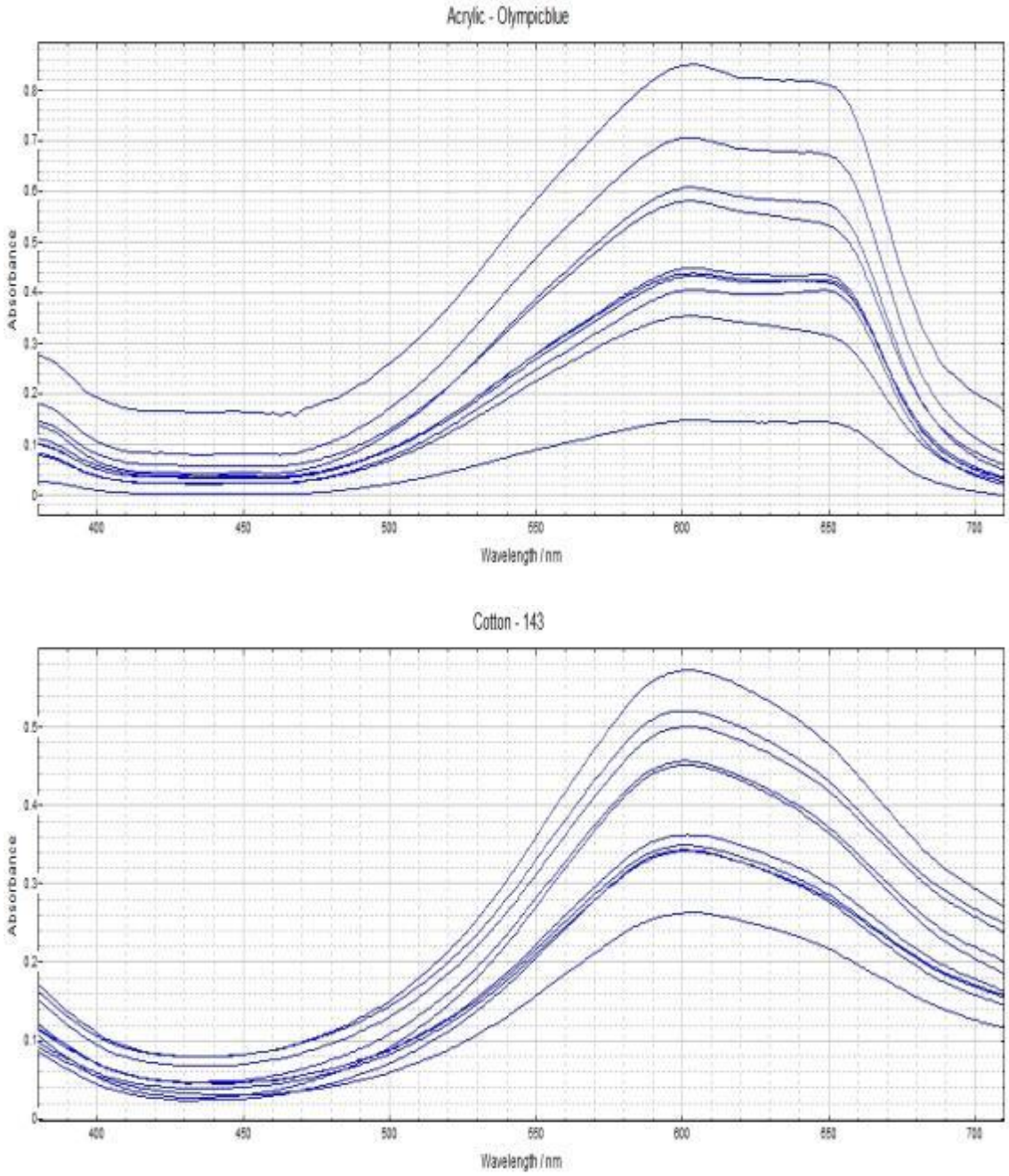

Figure 10: Demonstrating the spectral differences between two visually similar blue samples; acrylic "Olympic blue" (top) and cotton "143" (bottom). Each 10 fibres are from the same source – showing intra sample variability

Initially, visible range (380-710 nm) MSP was used for the analysis of the textile fibres. However, the wavelengths being used for obtaining the spectra can be increased to include the UV range, which may reveal further spectral information to allow for the successful discrimination of fibres from different sources [59,61,69]. When using UV-vis range MSP analysis in this research, the range of

wavelengths was increased from 380-710 nm to 280-710 nm. Anything below 280 nm was found to produce noisy spectra and therefore was not considered. UV was only considered in later experiments as it would be advantageous to develop a system that works with visible range MSP first and foremost due to the reduced equipment and consumable costs [4].

2.2.1 Collecting visible range (380-710 nm) spectra

Fibres to be examined were mounted on glass slides and placed onto the microscope stage and the settings in Table 4 used for acquisition. These settings were based on the standard operating procedures (SOP) provided for use alongside the MSP equipment and the ONYX software. The integration time in particular can be varied depending on the time pressures on the analyst and the quality of spectra required. A shorter integration time means that, in theory, a greater number of fibres per hour can be analysed as the acquisition of each spectrum will take slightly less time – thus increasing efficiency and throughput but potentially reducing the quality of the spectra obtained. The integration time used in this research allowed for high quality spectra to be obtained as time pressures were less so than would be expected in a working forensic laboratory which could opt to utilise a shorter integration time.

Table 4: Acquisition settings for visible range microspectrophotometry

Scan Type	Absorbance
Start Wavelength (nm)	380
End Wavelength (nm)	710
Integration Time (ms)	350
No. of Averages	10
Binning	1
Dark Spectrum	Selected

For spectrum acquisition, the measurement window was aligned alongside the fibre to be analysed in a north-south orientation to prevent any dichroism effects (i.e. differences in colours as a result of the fibres polarising) [43,68,123], whereby different colours and spectra can be observed based on the orientation of the fibres, and the fibre brought into focus on the monitor (Figure 11). Given the fact fibres tend to be birefringent, they must be oriented parallel to the direction of vibration caused by the polariser. In many laboratories the orientation of the polariser, the fibre and the diaphragms is standardised to the north-south direction [4]. This is because fibres exhibit pleochroism, where a variation in colour of the fibre is based on its orientation under polarised light [4]. Fibres act microscopically as “anisotropic uniaxial crystals” and can exhibit two such colours when in the parallel and perpendicular orientations due to the fibre’s and dye’s orientations – and are therefore termed “dichroic” [4]. Dichroism of fibres by MSP has been studied extensively researched by De Wael and Vanden Driessche [124,125], De Wael and Lepot [123,126] and De Wael [43] utilising polyester, polyamide, wool, silk, cotton, viscose, acrylic, acetate and pigmented fibres and found varying degrees of dichroism – for example strong dichroic effects were found in polyester

fibres [124], acrylic fibres showed limited dichroism which was hard to observe by light microscopy (but could be detected by MSP) [43] and in cotton dichroic effect was dependant on the chemical structure of the colourant [123].A background scan, including a dark spectrum scan, was acquired as per the above settings in Table 4.

For visible range MSP, the number of counts (which measures light intensity) was ensured to be 50000 +/- 5000; allowing for 10% leeway that would otherwise result in a large amount of fine tuning of the instrumentation before each analysis that would make the process incredibly time consuming and inefficient. In extreme cases, if the minimum number of counts was not achieved, the integration time was increased to increase the amount of light being captured and the above steps repeated until the number of counts was in an acceptable range as above. Keeping within this range of counts for all fibres being examined ensured that the information being obtained would be as robust as possible and attempted to ensure as many variables as possible were unchanged.

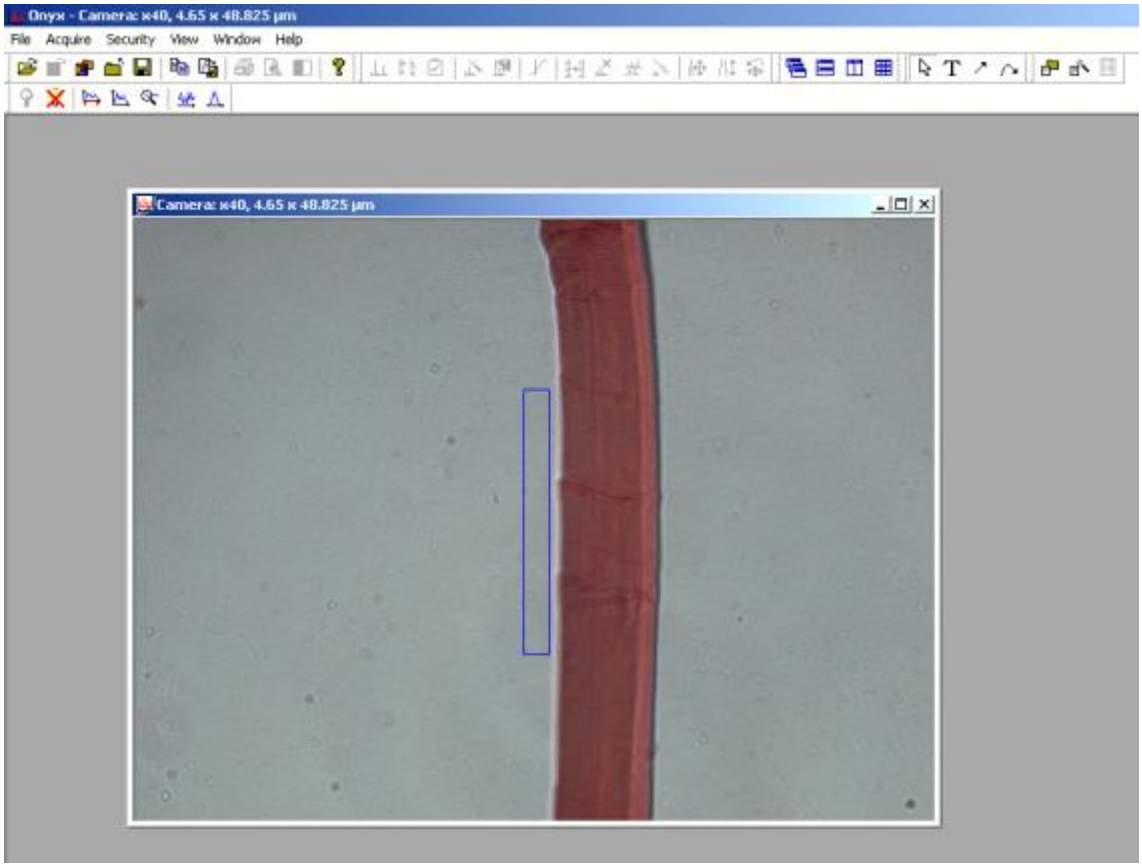

Figure 11: Example of the ONYX window, with a fibre to be examined in the visible range

Background scans were taken after any changing of focus [65] or light intensity settings (e.g. integration time) to ensure that spectra obtained were more reliable and had as little background interference as possible to avoid noisy spectra which can be harder to compare and interpret [3,53,65]. The measurement window was then moved to the centre of the fibre by adjusting the position of the microscope stage, ensuring to avoid areas of twisting or damage on the fibres per the European Textile and Hair Group recommended guidelines [41]. The obtained spectrum was then given a unique file name and saved. This process was then repeated three times along the length of the fibre. The three obtained spectra were then overlaid on the ONYX software and an average spectrum produced to intra-sample variation as discussed above. The above procedure was then repeated for each of the fibres being examined.

2.2.2 Collecting UV-vis range (280-710 nm) spectra

Fibres to be examined were mounted on quartz slides and placed onto the microscope stage and the settings in Table 5 used for acquisition. Again, these settings were based on the in house SOP provided for use alongside the MSP equipment and the ONYX software (see appendix1).

Table 5: Acquisition settings for UV-vis range microspectrophotometry

Scan Type	Absorbance
Start Wavelength (nm)	280
End Wavelength (nm)	710
Integration Time (ms)	350
No. of Averages	10
Binning	1
Dark Spectrum	Selected

For spectra acquisition in the UV-vis range, the measurement process was the same as for visible range MSP above, but with the following changes:

- The number of counts was ensured to be 20000 +/- 2000 (for reasons stated above with visible range MSP in terms of practicality and efficiency)
- The wavelengths examined were increased to 280-710 nm to include the UV range.

2.3 R

2.3.1 Importing spectra data to R

The spectra required for subsequent MVA and machine learning using R version 3.2.0 (R Core Team) were first opened in the ONYX software. The data was copied from the active spectra into Excel 2010 (Microsoft) and was transposed when pasting into Excel to ensure that each column represented a different variable and each row represented a different sample as required for efficient analysis by R. The file containing the data was then given a unique file name and saved in comma delimited (.csv) format to allow it to be subsequently read using the R scripts (see appendix 2).

2.3.2 Required R Packages

Two packages, used as PCA calculators, were required to be installed within R before the proposed scripts could be successfully run; “MASS” and “psych”. The “MASS” and “psych” packages were installed from the “Repository (CRAN, CRANextra)” library. Any additional dependencies were also installed automatically. When using these packages, the only default setting that was altered outside of those specified by the scripts was “tol” within the *lda function*. tol is the *“tolerance to decide if a matrix is singular; it will reject variables and linear combinations of unit-variance variables whose variance is less than tol^2” as a default”* [127]. tol was increased to six decimal places (by changing tol^2 to tol^6) as some values were very small and therefore would have been counted as zero by R - resulting in an error being displayed as the data appeared to be constant when in fact small changes in data were just not being detected by the pre-set tol^2.

2.3.3 R scripts and functions for performing MVA

The first script, “computeSPP”, was used to calculate the self-predictive probability (SPP) value for each fibre. This script was used for acrylic and cotton fibres, using “single source” or “pairwise” setting and with any number of total fibres (with the range determined by the number of samples in the dataset). “computeSPP” then called on two functions, “PCA_LDA_comb” and “LDA_own”. For both “PCA_LDA_comb” and “LDA_own”, the data was scaled and centred to give a variance of 1 and a mean of 0 respectively to avoid issues from disproportionate data and allow for analysis by linear discriminant analysis (LDA).

For “PCA_LDA_comb”, the dataset was then reduced using principal component analysis (PCA), with the number of principal components (PCs) to be retained being determined using the Kaiser Criterion (whereby PCs with an eigenvalue greater than 1 were retained). LDA was then performed using leave one out cross validation (LOOCV) on the retained PCs to calculate the self-predictive probability (SPP) values for each comparison. These concepts are explained in greater detail in the following chapter.

“LDA_own” works similarly to “PCA_LDA_comb”, but without the level of dimension reduction demonstrated when using PCA in conjunction with the Kaiser Criterion – i.e. only keeping components with an eigenvalue greater than one [79]. The maximum number of discriminate functions that can be created for subsequent classification is the number of variables minus 1, or the number of different groups - whichever is smaller.

In the “computeSPP” script the following information was input by the user:

- the path to the .CSV file containing the required dataset
- the total number of fibres to be used
- If single source or pairwise setting was to be used

For the single source setting, the total number of fibres was split into two equal sized groups 50 times – with each split containing a different allocation of fibres in each of the two groups (Figure 12).

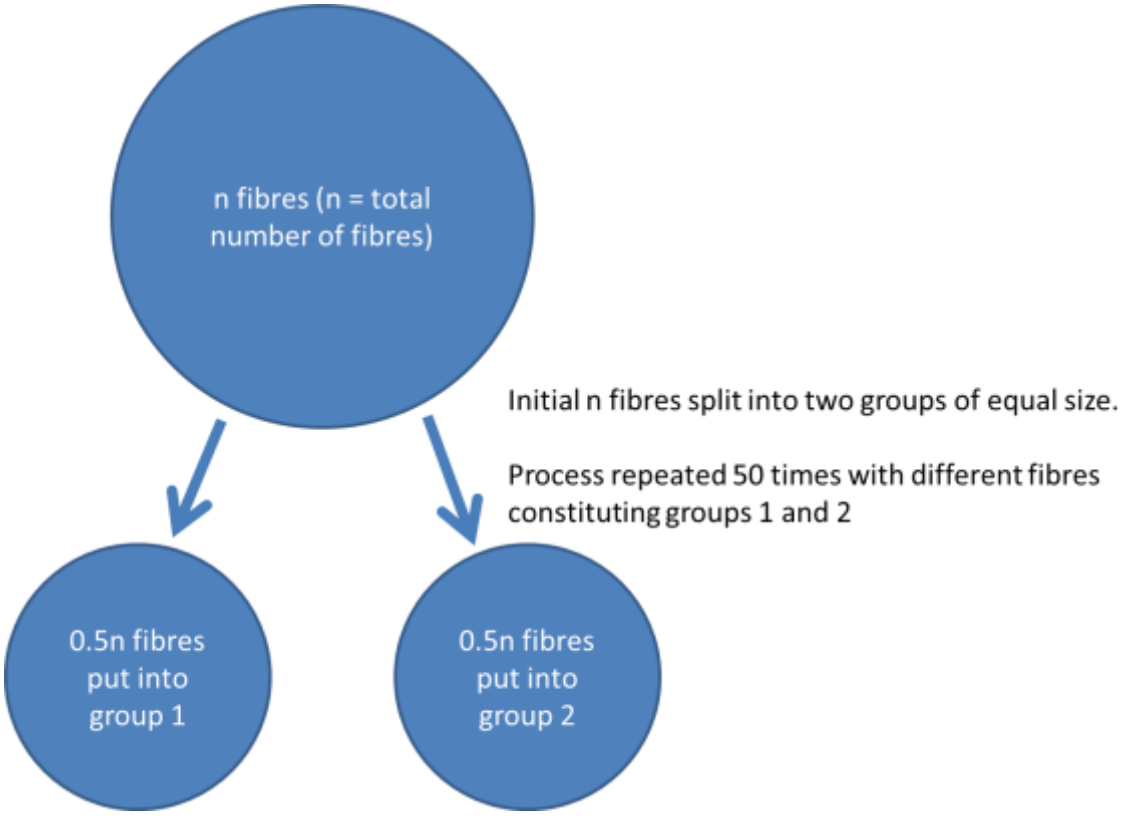

Figure 12: Visual representation of the fibres being split into two equal sized groups

For the pairwise setting, each group was compared against another group until all possible unique combinations had been exhausted. These pairwise combinations were constructed using the “*combn*” function within R. “PCA_LDA_comb” and

“LDA_own” were then both performed on the dataset, with the results from each input into storage arrays and saved to be used later.

Following “computeSPP”, the script “summary_decision_rule” was used. This script used the saved results from “computeSPP” to provide an output that can be used to compare the classification accuracy of each method. The fibre type, total numbers of fibres and setting (i.e. single source or pairwise) to be examined were input by the user so that the correct data file was used. In addition, the user specified whether the results when using PCA-LDA or LDA-own were to be displayed. The upper/lower SPP thresholds and exceedance proportions to be examined could also be altered by the user in the “summary_decision_rule” script to assess different values and the effect of these on recommendation accuracy.

“summary_decision_rule” then called on the “*decision_rules*” function to create the three groups for classification; different, indistinguishable and misclassification. The proportions of these, as determined by the specified exceedance proportions, then determined if the groups were “excluded”, “indistinguishable” or if “no recommendation” could be given and interpretations made.

The full scripts and functions used for MVA using R are provided in appendix 2.

2.4 Validation of the microspectrophotometer (MSP) and multivariate analysis (MVA) standard operating procedure (SOP).

In forensic science there will always be uncertainty in any measurement, and for any form of analysis to be suitable for application to case work the sources of uncertainty need to be known and, where possible, be reduced to allow for robust analysis to be performed and subsequent comparisons to be made [73]. Specifically, for the scope of this research in dealing with textile fibre dyes and subsequently MSP, sources of uncertainty would include inter- and intra- sample variation present in textile fibres as well as the data collected using MSP through its calibration and the settings used for spectra acquisition.

To reduce and control these uncertainties, documents such as the European Textile and Hair Group (ETHG) Fibre Examination Guidelines [41] exist - alongside standard operating procedures (SOPs) and other researched validations concerning data collection by MSP [65,128]. SOP documents can be created and made available to operators to ensure replicable use of equipment and their use is encouraged by the Forensic Science Regulator [39]. The above types of documents and reference materials assist the analyst in performing a robust examination with regards intra- and inter- sample variation e.g. by outlining how many fibres should (at minimum) be analysed for various fibre types (i.e. natural or synthetic) to help ensure a representative example has been examined by allowing for differences in dye uptake for example to be taken into account [29,58,85]. For instrumentation uncertainty, ensuring that the MSP has been serviced and calibrated using standards in line with recommendations can ensure that measurements are more robust i.e. likely to have the same outcome if the

same fibre was analysed in a different laboratory, but using the same equipment and procedure.

The protocol used in this research for the MSP was based heavily upon the SOP provided to the author at the commencement of the project (see Appendix 1). The MSP was serviced annually by a trained engineer. During this annual service all calibration checks were performed by the engineer and bulbs replaced as per recommended practice. During day to day operation of the MSP, methods were followed as outlined previously. During the collection of the data for this research, no unusual behaviour or suspected drift was observed from the MSP that could not be explained or remedied. All spectra to be directly compared were collected under the same (or as close as possible) conditions.

This section of the thesis will, where possible, outline the research behind the SOP and another other decisions made during the research as to allow for transparency and contribute towards the robustness of the proposed research.

2.4.1 The use of Microspectrophotometry (MSP)

Since Laing *et al.* [87] reported the use of MSP to discriminate visually identical fibres based on their visible absorption spectra in 1986, the application of MSP to fibre analysis has emerged as an indispensable tool for reliable fibre identification in forensic investigations [1]. MSP is crucial to the comparison process for textile fibres because it can segregate coloured fibres that appear visually the same but are subtly different (i.e. displaying metamerism). Objectively distinguishing between otherwise physically identical fibres is necessary to ensure a reliable comparison method. As well as being objective, MSP readings are repeatable, the

results are quantitative, and the methods can be standardised [3]; all of which are desirable features when considering the requirements of an ideal and objective methodology.

A previous study performed by the European Fibres Group (EFG now called European Textile and Hair Group, ETHG) and the Scientific Working Group for Materials (SWGMAT) Fibres section in the USA investigated if different MSP systems around the world would produce comparable dye spectra for fibres from the same source. In total 42 laboratories from 21 different countries took part in the project. The results of the study demonstrated that the tested MSP systems produced reliable and comparable dye spectra [4] and as such MSP is viewed as a gold standard for forensic analysis of textile fibre dyes.

2.4.2 Visible (Vis) range and UV-vis range MSP

Visible light (~ 360 – 780nm) refers to the region of the electromagnetic spectrum to which human eyes are sensitive; however since the sensitivity of the eye to radiation is very low at each of these extremes, in practice the visual spectrum is commonly taken as 380 to 720 nm [12]. Although there appears to be some inconsistency across previous research as to an exact upper and lower wavelength to be used for fibre analysis, almost all experiments tend to use wavelengths close to these values when considering analysis in the visible range. UV and visible range MSP have both been employed to analyse fibres previously [53,54].

When examining fibres in the visible range, glass slides and coverslips (alongside a suitable mounting medium such as phytohistol [118,120] or Entellan [119]) will often suffice; provided questioned and known fibres should be mounted using the same medium [4]. However, if measurement in the UV region is necessary, the use of quartz slides and cover slips is necessary, as well as non-fluorescent glycerol as a mounting medium [4].

From a scientific and data driven point of view, spectra utilising the full UV-vis range will generally provide more spectral information and consequently is likely to enhance the discriminating power [22,62,91,92] with Robertson, Roux and Wiggins estimating that about 10% of fibres examined that are similar in the visible region differ in the UV region [4]. However, it does not follow that the full UV-vis region must always be measured – for example, some fibres such as polyester absorb UV light [16,68] and would therefore not be suitable for UV analysis; or simply there may be enough information in a visible range MSP spectrum to exclude two samples from different sources. The financial aspects of recording spectra in the full UV-vis region must also be considered; as the need for optics transmitting UV radiation substantially increases system costs (as well as quartz slides and coverslips) compared to those for use in the visible region only [4]. Therefore, many of the experiments in this research were considered using only visible range MSP from 380 – 710 nm.

2.4.3 MSP set up prior to recording spectra

Prior to collecting any data using the MSP, as per the ONYX SOP (see Appendix 1) as well as other printed documentation [121] the MSP was switched on and the

lamps allowed to warm up for a minimum of 30 minutes before ensuring Kohler illumination.

2.4.3.1 Fibre orientation

Fibres are birefringent. Some of them, such as cotton, even show extreme polarising effects which may cause serious artefacts in the spectra [4,123]. This, as well as the polarising effects in the MSP itself, makes it necessary to use a polariser which must be placed in the front of the object [4]. Because the polariser produces linear polarised light, i.e. light with only one direction of vibration, the fibre must be oriented parallel to this direction. In many laboratories the orientation of the polarizer, the fibre, and the diaphragms is therefore standardised to the north-south direction [4] and this was reflected in the SOP document provided for use alongside the MSP used in this research.

2.4.3.2 Measurement window shape and placing the measurement window

Fibres are linear objects; meaning a long, narrow rectangular shape is the most suitable one for the measurement slit [4]. When collecting spectra, the measurement window was placed within the fibre at the approximate centre – ensuring to avoid areas of damage or twisting where possible. This location can be seen demonstrated in the top left of Figure 13 below.

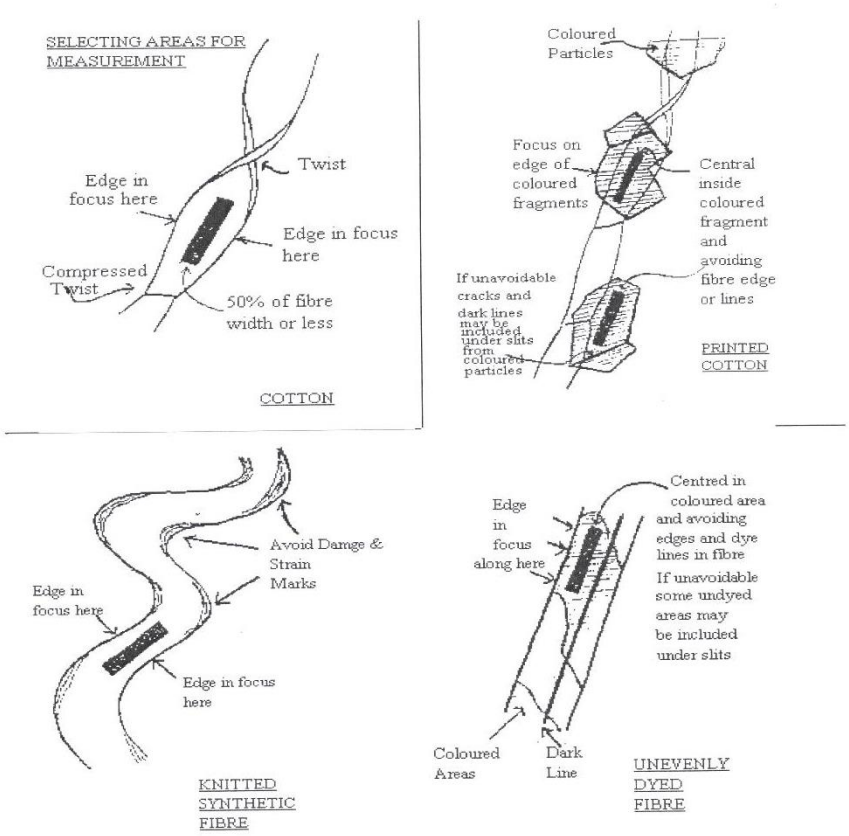

Figure 13: Diagram showing the areas to select for MSP measurement for various scenarios [121]

From a data collection and interpretation standpoint, spectra collected through the relatively broad, flat portions of cotton fibres should not be compared to spectra collected through any twisted areas. Similarly, spectra collected through the projecting side lobe of a trilobal fibre should not be compared to spectra collected through the vertical central lobe [65]. Therefore, it was ensured that all data collection was performed on flat areas of fibres which showed no damage or artefacts

2.4.3.3 Light "signal" reaching the detector

The amount of light reaching the detector was monitored by using "counts" wherever a new slide was placed on the stage. For visible range MSP the counts

were ensured to be 50,000 +/- 10% when the measurement window was placed alongside the fibre to allow for good quality spectra to be obtained. For UV-vis range MSP, the counts were ensured to be 20,000 +/- 10%. The counts were observed in real time when each new slide was placed on the stage and small adjustments were made to signal strength by raising or lowering the substage condenser rack on the MSP. Key Forensic printed documentation [121] states that higher and lower counts are acceptable as long as these are not so low that noise levels become significant or so high (~60,000) that saturation occurs.

Palenik *et al.* [65] suggest that substage aperture can be opened to varying degrees to alter the amount of light reaching the detector; and in some instances it may be necessary to open this further to increase the amount of light reaching the detector. However, the substage aperture was left in a fixed position during the course of the experiment to ensure that any spectra compared to each other were collected under the same conditions as possible.

2.4.3.4 Acquisition settings

Based on the provided SOP for the MSP, the default settings for spectra acquisition in the visible range are:

Scan Type: Absorbance

Start Wavelength: 380 nm

End Wavelength: 710 nm

Integration Time: 600 ms

No. Averages: 10

Binning: 1

Dark Spectrum: selected.

For UV-vis spectra acquisition, the same settings as listed above were used, but with a lower start wavelength of 280 nm.

For comparison, a document produced by Key Forensic [121] lists the default settings for spectra acquisition in the visible range as:

Scan Type: Absorbance

Start Wavelength: 390 nm

End Wavelength: 700 nm

Integration Time: 100 ms (this can be increased if dealing with pale fibres or small samples)

No of averages: ~4-10

Binning: 1

Dark Spectrum: selected

As can be seen between the two recommendations many similarities are present, with small differences being observed in wavelength range and a larger difference being observed in integration time – but with a note in the Key Forensic document that their stated value may be increased.

Absorbance was utilised for scan type as it is directly proportional to concentration of dye in the fibre [65]. When two fibres from the same source are dyed with the same dye but one fibre takes up/retains more dye than the other, differences will be evident in their absorbance spectra. However, the shape of the spectra will generally be similar [53].

The wavelength ranges are based on the typical visible or UV-vis ranges and will depend on fibre type, information required, and mounting materials (mountant, slides, coverslips) used.

Most MSP systems allow the user to define the sampling time and the number of scans to average. Palenik *et al.* [65] state there are no rules dictating the values of these parameters but that their values will be determined by the sample being analysed and they should be adjusted until high quality spectra are produced. They do however note that that long sampling time may saturate the detector, resulting in unusable spectra. They also recommended that all of the data within an experiment be collected using the same conditions and so the integration time (350 ms) and number of averages (10) was kept constant where possible.

Binning, as defined by the ONYX manual [129] is “the number of CCD pixels that are binned together on the spectrometer. The higher the number the lower the resolution” so it makes sense to have this number at a low value of 1 to ensure high resolution.

Dark spectrum (i.e., when the light from the microscope is blocked from the detector) scans were collected throughout the course of data acquisition. Palenik *et al.* [65] recommend collecting reference scans before every sample scan is collected. This high frequency of reference scan collection compensates for the effects of adjusting the fine focus on the microscope as various portions of the sample are brought into sharp focus and so this was performed during this research. They also recommend collecting dark scans after changing slides; practice which was again implemented.

2.4.4 Number of fibres per sample

The environment the dye finds itself in, i.e. the textile fibre, has some influence on the shape of the spectrum produced [65] and therefore carries practical consequences [4]. Synthetic fibres such as polyester or acrylic usually have a more homogeneous chemical constitution compared to natural fibres such as cotton or wool. The dye in synthetic fibres is bound to a relatively constant chemical environment, whereas natural fibres are composed of many different chemical components which are inhomogeneously distributed throughout the fibre matrix. As such, colour may vary along the length of a fibre due to differential dye uptake, particularly in natural fibres [3,29,58,85]. Therefore, spectra of dyed synthetic fibres usually show less intra-sample variation with respect to the wavelength position of the absorption bands compared to those from natural fibres; meaning that it is normally necessary to measure more natural fibres to get an overview of the spectral variation within the sample [4,12].

Typically, ten natural fibres and five synthetic fibres would, at minimum, be the number normally measured [4,41,58]. Some previous works, such as those by Wiggins *et al.* [58], used synthetic fibres when testing their methods as these were viewed as “the least problematic samples” from a casework perspective. However, as one of the goals of this research was to establish a set of criteria that could be applicable to all fibre types dyed cotton fibres were used to investigate the effect of number of fibres measured on the classification accuracy of the MVA model as these typically require more spectra to be taken compared to synthetic fibres. Red, blue and black cotton blocks of colour were analysed using visible range MSP, alongside the “LDA-own” MVA approach to investigate the effect of number of fibres per group on classification accuracy (Figure 14).

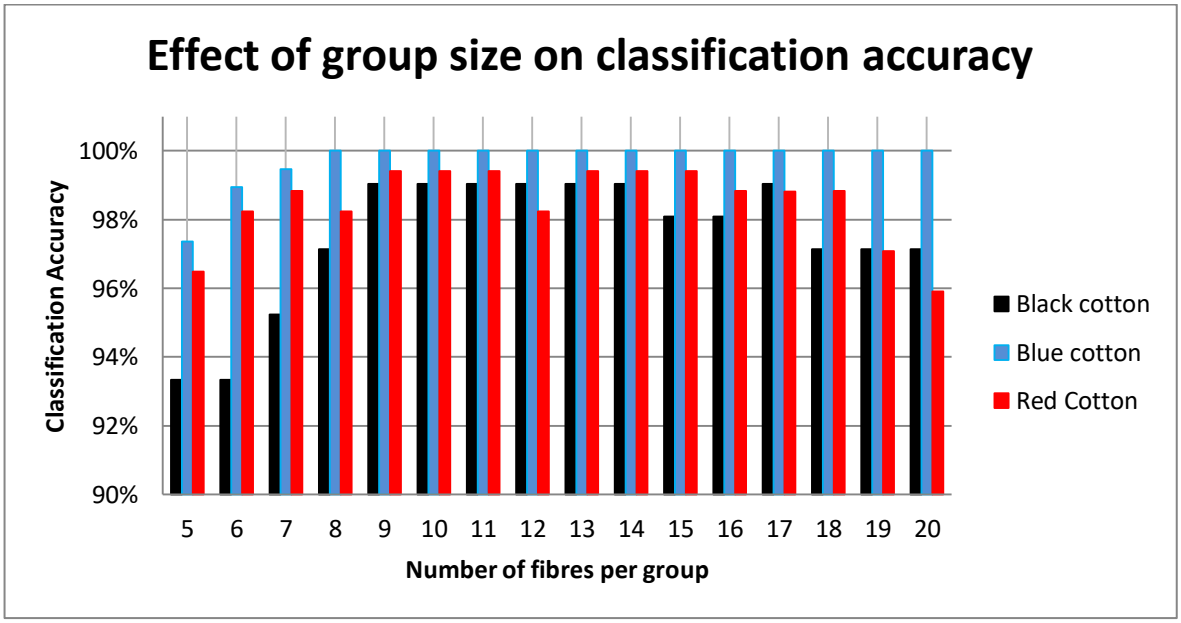

Figure 14: The effect of number of fibres per group on classification accuracy when using cotton blocks of colour

Figure 14 shows that for each of the red, blue and black cotton blocks of colour, the maximum classification accuracy (red ~99.4%, blue 100% and black ~99.1%) was observed when 9, 10 or 11 fibres per group were used. Using only 5 fibres per group for red, blue and black cotton fibres resulted in ~96.5%, ~97.4% and 93.3% accuracy respectively. As such, this enforces that using 10 fibres per group may be used for the experiments as this a) produced the joint best results in the above test and b) was in keeping with the previously established recommendations in forensic science.

As a final note on this section, logically using 10 fibres per groups gives better classification accuracy compared to 5 fibres per group as there is more information present for the model to accurately differentiate samples. Therefore it would follow that more information, in the form of more fibres scanned, would continue to improve or maintain accuracy; however this was not observed. As shown in Figure 14: The effect of number of fibres per group on classification accuracy when using

cotton blocks of colour, the general trend shows a decrease in classification accuracy as the number of fibres per group exceeds ~16. The true cause of this was not investigated as it was not key to the research, but it is hypothesised by the author that excessive data available to the MVA model resulted in excess noise of overfitting occurring which ultimately confused the model; resulting in reduced classification accuracy. Furthermore, due to the nature of fibre persistence (i.e. that fibres are lost from surfaces over time) [21,130–135] a lower number (e.g. 10) rather than a higher number (e.g. 20) would be beneficial and more likely to be applicable.

2.4.5 Scans on each fibre

The location of the collection aperture (measurement window) relative to the sample should remain constant throughout any given experiment as to ensure representative comparisons; this position was demonstrated previously in Figure 13. This is especially important for fibres with complex or irregular cross-sections. However little guidance is provided as to how many scans along the length of each fibre should be taken, or where to scan (outside of being in a consistence place anyway from damage and debris). It has been noted that even organisations such as SWGMAT do not mention how many areas along a single fibre should be analysed and that similarly the European Fibres Group states that it is “not recommended to set a definitive rule on the numbers of fibres that should be analysed” [65] . Some examples of previous suggested/utilised approaches are listed below in

Table **6**.

Table 6: A summary of some previously suggested or utilised methods for number of scans per fibre

Authors	**Year**	**Suggested or utilised approach for number of scans per fibre**
Deviterne-Lapeyre *et al.* [136]	2012	Three, but spacing not specified, then two closest matches for the three plus an average
Palenik *et al.* [65]	2016	Minimum of three along the length
Sauzier *et al.* [71]	2016	Five along the length of each fibre
Reichard *et al.* [67]	2017	Five along the length of each fibre
Powell *et al.* [85]	2018	10 for synthetic, 20 for natural. Then three used for analysis; 1 average, 2 showing one and two standard deviations respectively

As demonstrated above, there is no real consistent or steadfast approach to the number of scans to be taken per fibre ranging from three up to 20; although most suggest these scans be taken **along** the length of the fibre. Some authors suggest that averages should also be used. The two studies above that have the same approach of five scans along the length of each fibre share a common co-author who may have brought this approach forward in both studies. Finally, it Is possible to compare individual spectra, but to do so is generally not recommended as this approach does not take into account potential intra-sample variation (unless multiple examples of individual spectra are compared between two, or more, items) [65] thus increasing the change of false exclusions.

With regards validation, this question of how many scans per fibre and where they should be taken from relates to the repeatability (i.e. scans in a single place on each fibre), reproducibility (i.e. scans along the length of each fibre) and robustness (the ability for similar results to be obtained by different analysts using a SOP) [128]. A small experiment was undertaken to compare the spectra produced using visible range MSP when taking six scans from a single location on a fibre compared to the spectra produced when taking three scans along the length of the fibre. Five cotton swatches (from different depths in the same dye catch of Direct Red 23) were selected from 24 swatches available. For further details on dyeing process, please see the own dyed section discussed later in this research. From each swatch a sample was taken in the same way as the other visible range MSP experiments; taking a scraping of fibres and mounting onto glass slides in phytohistol before applying a glass cover slip. Six fibres were examined using each approach, three scans along length and six scans in one place before averages were taken and compared. The averages from each six fibres in the swatch using both approaches, as well as the overall average from each approach in each swatch are shown in the figures below; with the average for each approach for all analysed swatches being shown in Figure 20.

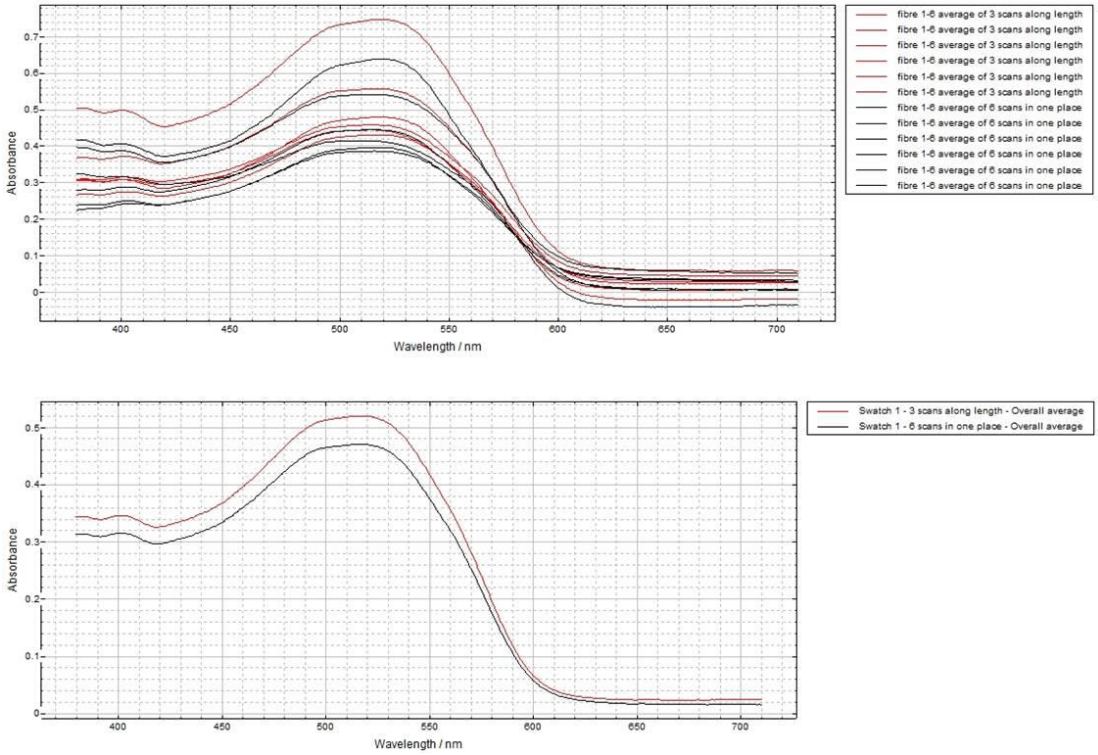

Figure 15: (Top) The averages from each of the six fibres in swatch 1 using 3 scans along the length (red) and 6 scans in one place (black). (Bottom) The overall average from the six fibres in swatch 1 for 3 scans along the length (red) and 6 scans in one place (black)

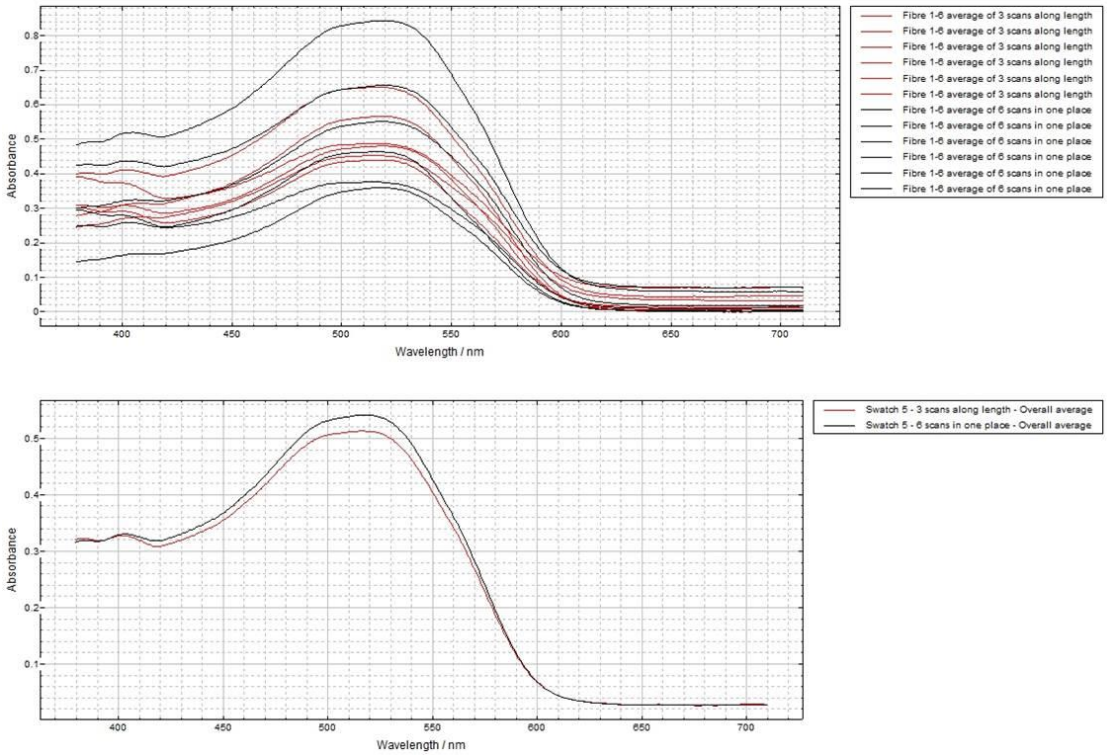

Figure 16: (Top) The averages from each of the six fibres in swatch 5 using 3 scans along the length (red) and 6 scans in one place (black). (Bottom) The overall average from the six fibres in swatch 5 for 3 scans along the length (red) and 6 scans in one place (black)

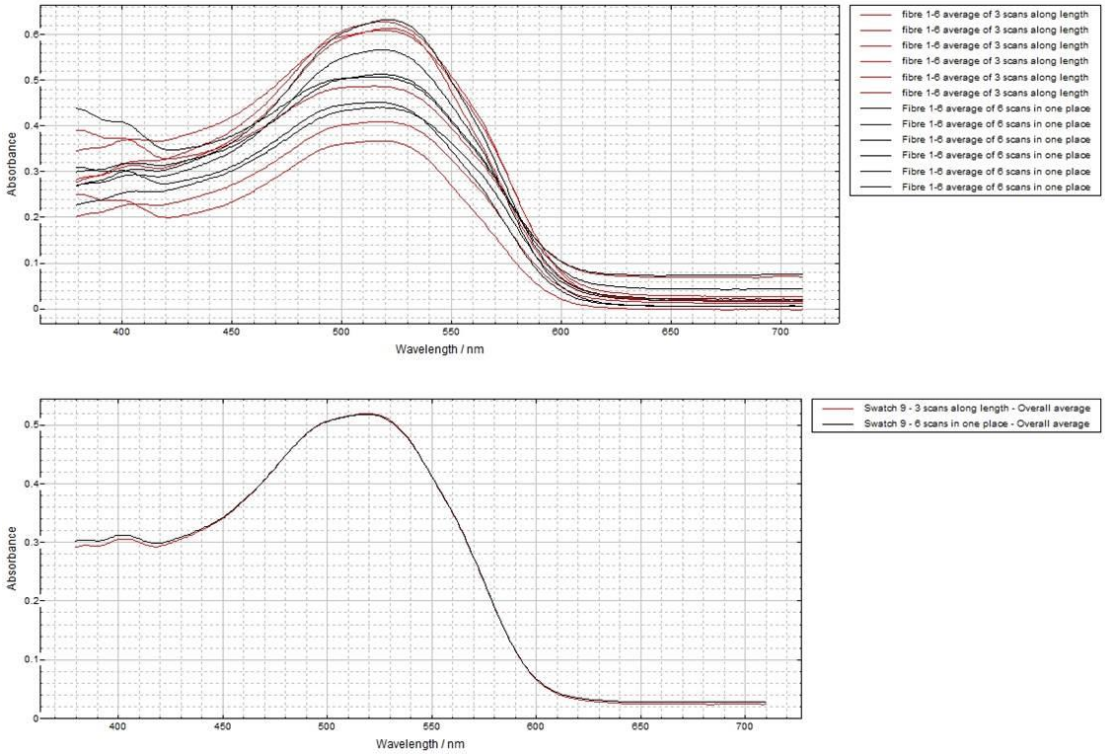

Figure 17: (Top) The averages from each of the six fibres in swatch 9 using 3 scans along the length (red) and 6 scans in one place (black). (Bottom) The overall average from the six fibres in swatch 9 for 3 scans along the length (red) and 6 scans in one place (black)

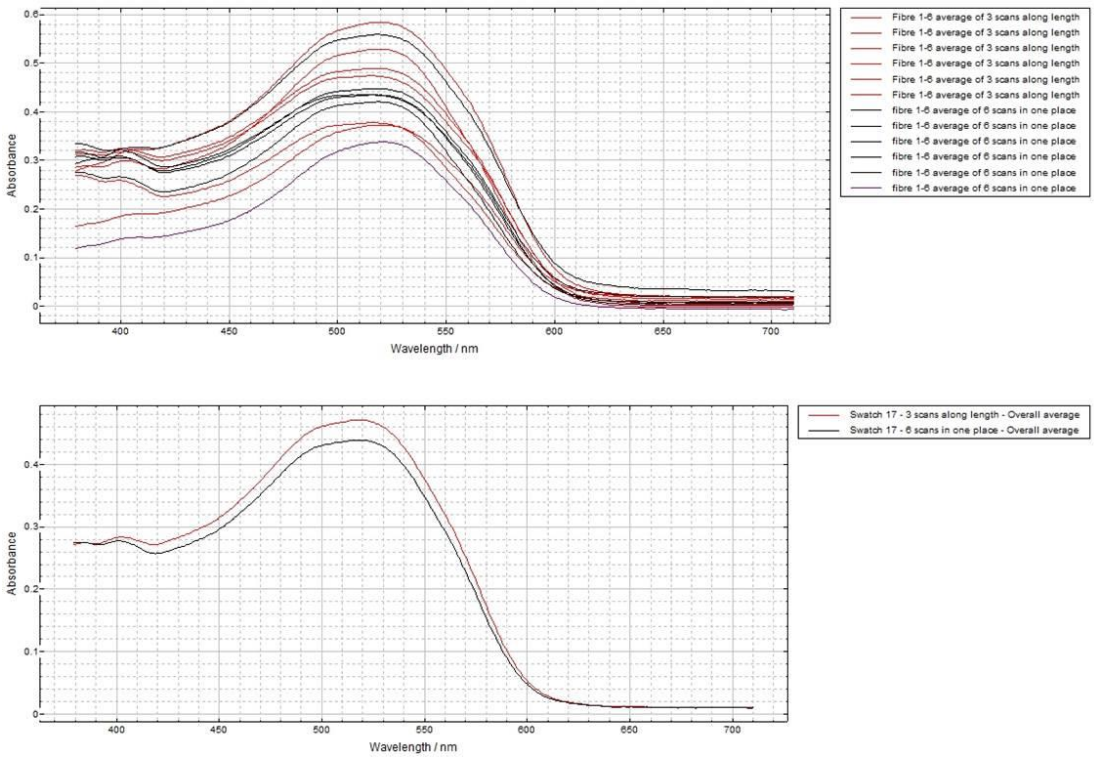

Figure 18: (Top) The averages from each of the six fibres in swatch 17 using 3 scans along the length (red) and 6 scans in one place (black). (Bottom) The overall average from the six fibres in swatch 17 for 3 scans along the length (red) and 6 scans in one place (black)

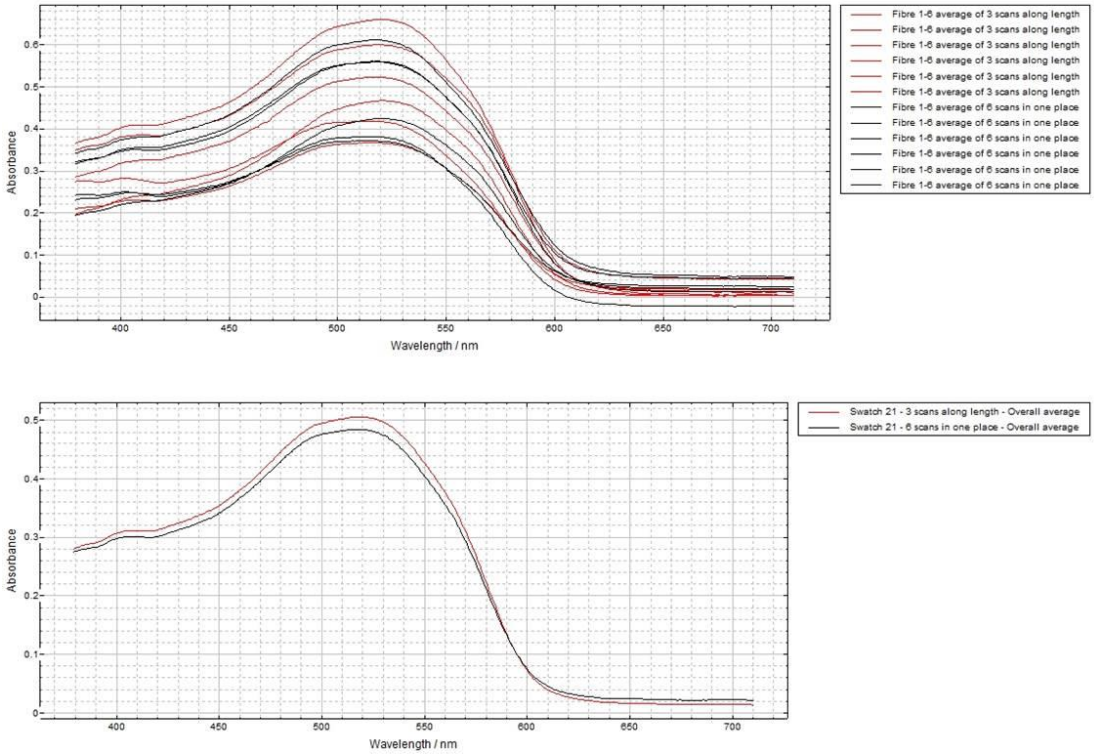

Figure 19: (Top) The averages from each of the six fibres in swatch 21 using 3 scans along the length (red) and 6 scans in one place (black). (Bottom) The overall average from the six fibres in swatch 21 for 3 scans along the length (red) and 6 scans in one place (black)

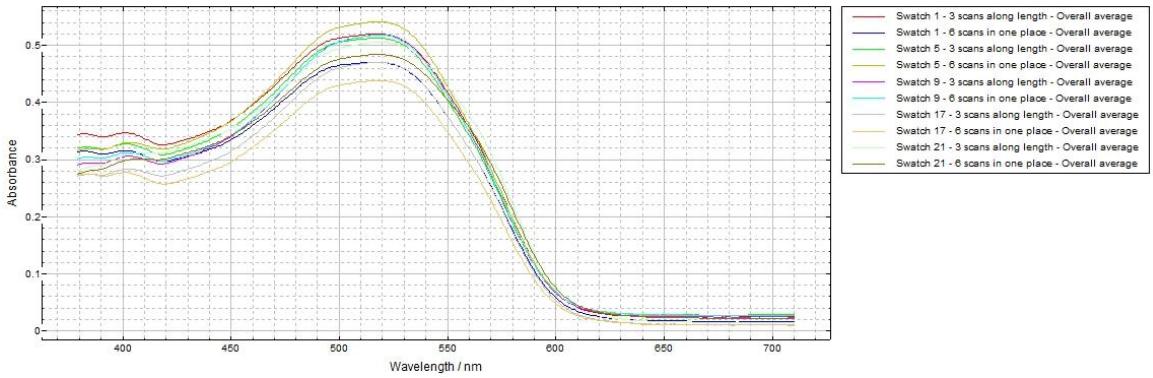

Figure 20: The overall average for each analysed swatch using both 3 scans along the length and 6 scans in one place

From the spectra presented above, it can be seen that there is no notable difference in spectra produced regardless of if using three scans along the length of each fibre compared to six scans in one place. Therefore, it is suitable to utilise three scans along the length of each fibre as proposed in the methodology and as suggested by Palenik *et al.* [65].

3. Developing an Ideal Classification System

3.1 Purpose of this research

There has been limited work published to date regarding the application of multivariate analysis (MVA) and machine learning to textile fibres [67,71,136,137], with only one of these looking at a practical “questioned vs. known” scenario [71] whereby a question of match/non-match was considered i.e. were samples that were considered to be indistinguishable and therefore potentially from the same source correctly recommended to be a “match” whereas samples that were considered to be different and therefore from different sources were recommended to be “non-match”. However, this paper only used one fibre type (acrylic) and 11 different sources – resulting in a potential sample size issue.

The aims of this research therefore are to provide a more comprehensive dataset to support the initial findings from other researchers as well as the novel methodology developed here.

3.2 Requirements of an "ideal" dye classification system

A proposed classification system has three requirements that need to be met in order be "ideal". It must:

i. Utilise a probabilistic approach
ii. Require minimal user input
iii. Be robust

3.2.1 The Probabilistic Approach

Probabilistic theory has been offered as a model for interpreting and evaluating forensic evidence previously for a variety of evidence types [33,36]. Those who have proposed the utilisation of a probabilistic approach believe it provides a model for incorporating the most relevant information available into an evaluation [4]. By utilising a probabilistic method, areas of uncertainty can be considered and addressed rather than using fixed, precise values that do not allow for error. This is important, not only given the "indistinguishable vs. distinguishable" question that is considered in this research, but the need for addressing uncertainty was also highlighted in the National Research Council report with reference to other evidence types such as fingerprints – that have the element of subjectivity in their analysis [31].

This research utilises a probabilistic approach by determining the probability of assigning a fibre to its true source rather than another, incorrect source – referred to hereon in as "self-predictive probability" (SPP). An arbitrary range of thresholds to be used in conjunction with the SPP value were decided on after evaluation of some preliminary data, in order to determine which threshold gives consistently high recommendation accuracy across a variety of scenarios – without the need

for continual user input and interpretation and manipulation of the thresholds. This then leads to the next criteria for an ideal system – minimising user input.

3.2.2 Minimising User Input

How much user input constitutes “minimal” is, in itself, subjective. However, considering the current MSP spectra comparison process performed by an examiner, where hundreds of absorbance readings across various wavelengths for each spectrum are considered, a reduction in user input may improve on efficiency, turnaround time, and contribute to a reduction in the number of potential disagreements occurring between experts.

The first stage of this research involves determining an “optimal” set of criteria (i.e. user inputs) through an extensive list of combinations of SPP thresholds and exceedance proportions (E.P.) alongside the number of fibres per group. The European Textile and Hair Group (ETHG) Fibre Guidelines [41] include the number of fibres that should be examined for different fibre types. Similarly during this research, a number of fibres per group will be established that allows for robust analysis and classification.

From a practical aspect, the smaller the number of fibres per group required, the better, as fibre transfer and persistence studies [131] demonstrate how quickly fibres can be lost from a substrate but also that a relatively small number of fibres may be transferred in the first place. Therefore, if only finite number of fibres transfer and persist, only a limited number will be available for subsequent analysis. However, a balance needs to be struck with keeping a small number

required, but also ensuring that a representative sample is used to allow for accurate classifications to be made.

This research allowed for the determination of the optimal settings allowing for high correct classification accuracy over a variety of scenarios and fibre type/colour combinations. Once the optimal settings had been established through a thorough set of experiments, these were fixed and utilised in further, increasingly challenging experiments and investigated to determine the classification accuracy which are more likely to be challenging to the fibre analyst.

The advantage of minimising user input is a point discussed later in this chapter, whereby some forms of multivariate analysis (MVA) require a different point at which the user would determine the threshold for inclusion or exclusion of a sample. This, in turn, introduces subjectivity [65] as well as potentially decreasing the overall robustness of a proposed technique as different users could obtain different results from the same dataset if differing settings and inputs are used.

3.2.3 Robustness

Robustness, in a statistical context, implies that a method should perform consistently under different situations; situations in which the method is designed for [128]. In the context of this research, robustness means the proposed classification system performs well across different colour pairs (e.g. red vs. blue, green vs. yellow, blue vs. blue etc.) and different fibre types (e.g. acrylic and cotton).

3.3 The Classification System

The key innovation of this research is the proposed classification system that can be applied to each casework situation. Given two groups of fibres, the system utilises MSP data to recommend whether the two groups of fibres are indistinguishable (and therefore may have originated from the same source) or distinguishable (and therefore originate from different sources). If the information contained in the two groups of fibres (in the form of data from the MSP spectra) is insufficient, the system will provide no recommendation as to whether the two groups of fibres are indistinguishable or distinguishable. The proposed system is different from a database approach, where one wants to know whether the groups of fibres being analysed match those in a previously constructed database or not.

There are five key aspects to this proposed classification system which are expanded on in this chapter:

1. Identifying appropriate multivariate analysis (MVA) methods
2. Defining and justifying the use of leave-one-out cross validation (LOOCV)
3. The application of a self-predictive probability (SPP)
4. The application of an exceedance proportion on which to base an overall recommendation
5. The three different recommendation categories (i.e. indistinguishable, excluded or no recommendation)

3.4 Multivariate analysis

3.4.1 The current situation for analysis of MSP spectra from textile fibres

MSP analysis uses the spectral information obtained over a range of wavelengths – in the case of this research from 280 - 710 nm for UV-vis range and 380 - 710 nm for visible range. The obtained spectrum contains not only information relating to intensity (i.e. the wavelengths where the maximum and minimum absorbance values are observed), but also information relating to the shapes of these maxima and minima, the shape of any shoulders present in the spectra and other features such as points of inflection or the gradient of different section of the spectra curve [40].

The current interpretation of these features is not defined by any mathematical procedure, but more so by the eye-brain system i.e. based on the experience, opinion and observations of the analyst. An overall correlation between all of the observed features, including general shape of the curve in its entirety, must be established by the analyst before a “match” can be concluded [40]. The control fibres are generally examined together first, to establish the standard range exhibited by the control fibre. A number of fibres deemed appropriate to ensure the variation within the sample is fully captured will be used. The number of fibres will often be determined by the fibre type or the variation present within the sample i.e. the number of fibres examined will generally increase as the dye variation increases, to ensure that all variation is suitably captured to ensure a more robust evaluation. Questioned fibres are then systematically compared to the control fibres to determine if the questioned fibre lies within the range observed with the control fibres and that all of the spectra structural details are indistinguishable [40].

3.4.2 Choosing appropriate multivariate analysis techniques

The above described visual and opinion based comparison of spectra shapes and details from the eye-brain system of the fibre examiner, demonstrate a subjective element: relying on the examiner's experience, observations and judgement. Although uncommon, this subjectivity can lead to disagreement between examiners as to whether or not two samples are indistinguishable and therefore may have originated from a putative source. The combination of the US National Research Council report [31] (amongst others, such as the Forensic Science Regulator's Report [39]) calling for the implementation of more robust and objective analysis, coupled with the recent influx of publications demonstrating the application of multivariate analysis (MVA) to forensic evidence [1,71,81,115,136,138] has led to this research.

Many papers involving the use of MVA in a forensic science context [1,67,71] have involved some, all or variations/combinations of principal component analysis (PCA) and linear discriminant analysis (LDA).

3.4.2.1 Principal Component Analysis (PCA)

Supervised MVA techniques use group information when creating data models, whereas unsupervised techniques only look at underlying structure in the data with no group information. PCA is an unsupervised technique. Unsupervised data mining does not focus on predetermined attributes, nor does it predict a target value. Rather, unsupervised data mining finds hidden structure and relation among data. PCA has two common applications - classification based on degree of similarity and dimension reduction [139].

Classification using PCA involves the user making subjective evaluations and interpretations as to how similar groups of samples are, when visually examining their position on 2D or 3D plots in order to group or cluster them. An example of a PCA utilised for classification and clustering from Sauzier *et al.* [71] is shown in Figure 21; where PCA was used to try and form different clusters for each fibre set. This figure demonstrates that if such a technique was to be used on samples of an unknown origin (i.e. in a known vs. questioned comparison) then the user could easily mistake groups that clustered similarly as being part of the same groups e.g. fibre sets C&D, E&I or A&H.

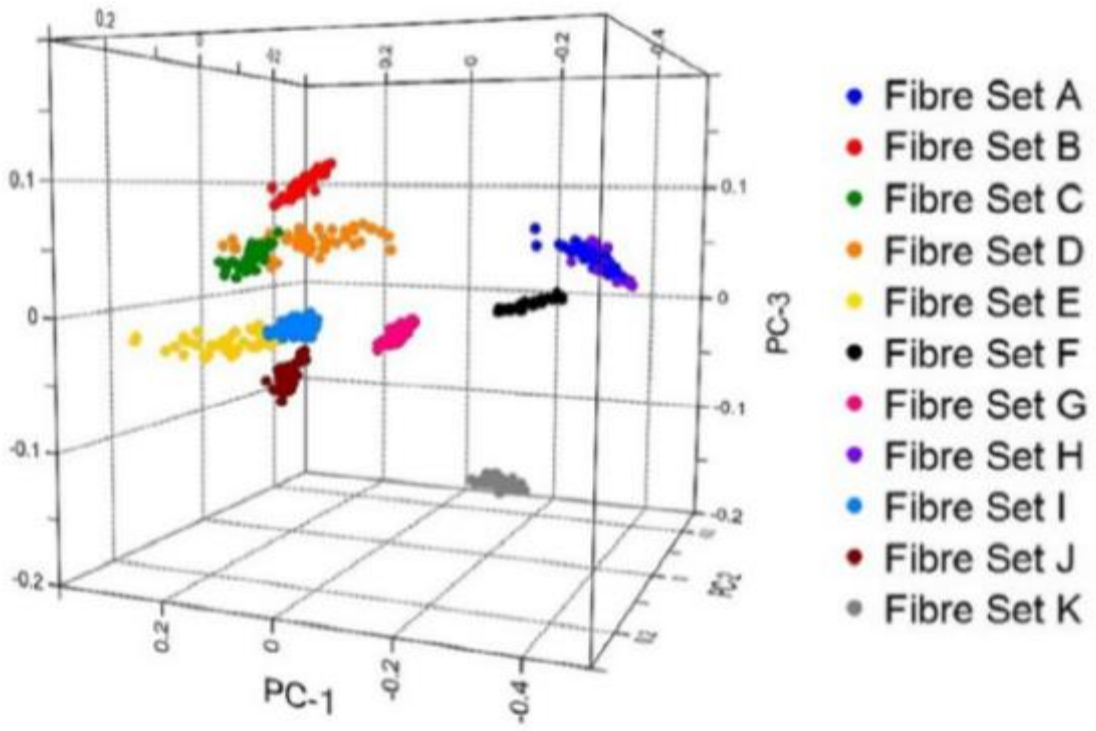

Figure 21: An example of clusters for interpretation when using PCA from Sauzier *et al.* [71]

This again reflects a situation that introduces subjectivity and lack of consistency between different datasets – exemplified by the statement by Palenik *et al.* [65] above. Based on the subjective nature of grouping using PCA for classification based on opinionative evaluation and interpretation, this application was not

considered suitable for this research. However, PCA as a dimension reduction technique prior to other analysis such as LDA is commonly utilised in published research [1,67,71] and was more suitable for application to this research.

Dimension reduction refers to the methods used to represent data using fewer columns or features and can be accomplished through unsupervised methods. This can provide a more objective method, as the process of determining which data to retain is based on mathematical calculations (utilising probabilistic method and minimising user input). The application of LDA requires more samples than variables [140] which is why it is often performed after using a dimension reduction technique such as PCA (termed PCA-LDA in this research). For this research specifically, this would mean that in order to be able to apply LDA without prior dimension reduction, over 404 fibres would need to be analysed in order to meet this prerequisite. This would be very time and resource intensive - and therefore not representative or practical for the forensic science community. However, when using the *lda* function in R, prior dimension reduction is also possible as n-1 canonical variates (CVs), where n = number of samples, are created if the number of samples exceeds the number of variables. This approach of not utilising dimension reduction through PCA, but relying on this inbuilt feature of *lda* is termed LDA-own in this research.

Prior to performing PCA for dimension reduction, the dataset was standardised to ensure an overall variation is equal to 1 and the mean is equal to 0. This was performed using mathematical calculations which can be automatically applied by R. The dimensionality of the dataset was then reduced by finding a smaller number of variables that explained the maximum variance with linear combinations of the original variable – demonstrated in Figure 22. These are called principal

components (PCs) [141]. The first PC explains the most variation within the dataset. The second PC then explains the next most variation that has not been accounted for by the first PC and so on. If there are *n* variables in the dataset, then there are *n* possible PCs; however the majority of the information relating to variation could be accounted for by the first few PCs – thus creating the dimension reduction capabilities of PCA. However, these PCs may be the most useful for describing variance, but not necessarily for classifying, as the two are not necessarily the same. This therefore means that there is the potential for information that would be useful for classifying two different groups of fibres (or any other sample) could be "lost" during this dimension reduction process.

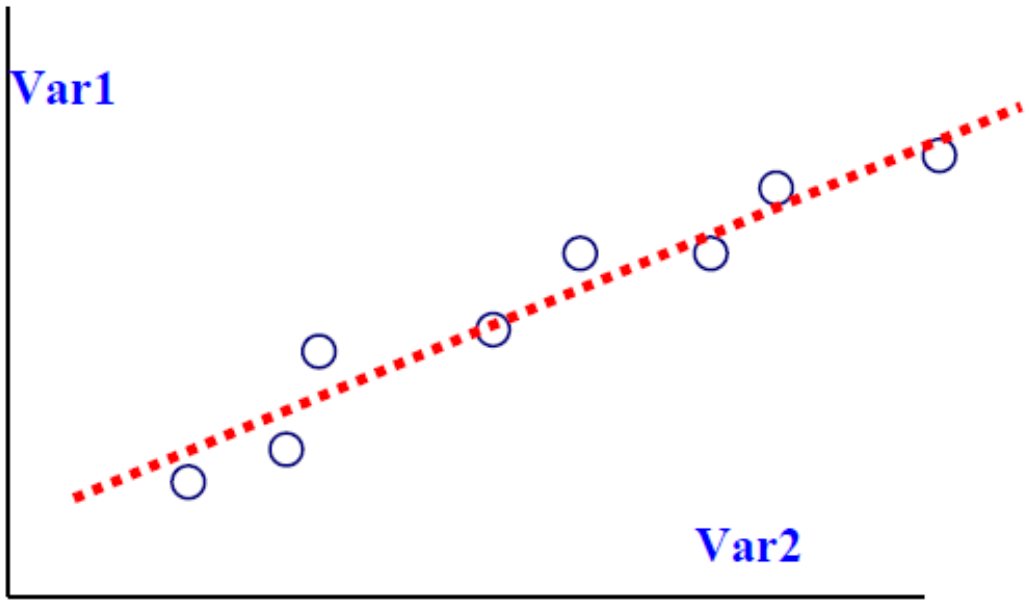

Figure 22: The Linear Combination of Two Variables, Var1 and Var2

Figure 22 shows that Var1 and Var2 are correlated, meaning that they provide a great deal of common information. Because they are correlated a single composite variable including both Var1 and Var2 could be used instead of using both Var1 *and* Var2. This composite variable would in fact correspond to the line in Figure 22

and would be a principal component (PC). In an actual dataset, there may be several sets of multiple correlated variables; hence several PCs could be constructed.

Generally, for n variables (Var_n), all extracted PCs will have the general equation shown in Equation 4 - where the coefficients a, b, c etc. are factor score coefficients.

Equation 4: The General Equation for Extracted Principal Components

$$PC_1 = a1_1*var1 + a1_2*var2 + a1_3+var3 + \ldots\ldots a1_n*var\ n$$

$$PC_2 = a2_1*var1 + a2_2*var2 + a2_3+var3 + \ldots\ldots a2_n*var\ n$$

The number of PCs to be retained for subsequent analysis can be determined one of two ways - either using a scree plot or using Kaiser Criterion. When using scree plots, visual examination is required by the user to determine where the “elbow” in a scree plot occurs. An example of a scree plot is shown in Figure 23.

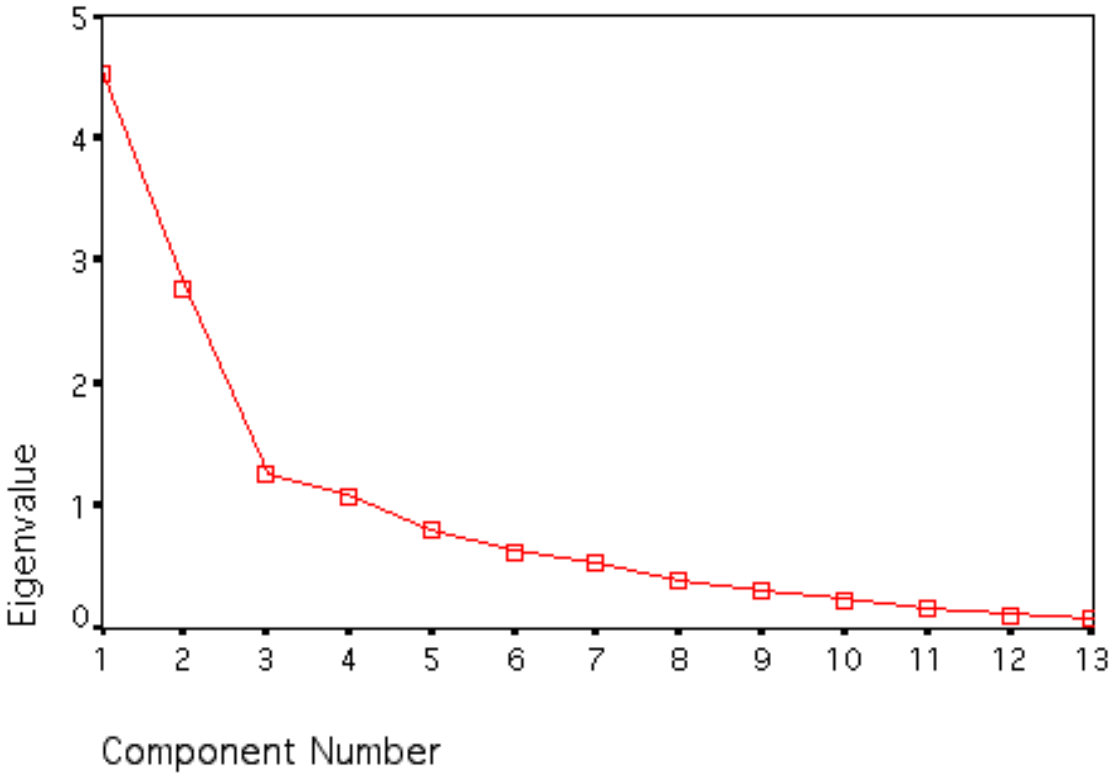

Figure 23: An Example Scree Plot [142]

Using the scree plot approach requires user input as to the interpretation of the produced plot, and would require re-evaluation after each analysis. Not all scree plots will be easy to interpret, therefore introducing an element of subjectivity. Therefore, a more objective method would be to use Kaiser Criterion.

Using the Kaiser Criterion, only PCs with an eigenvalue greater than or equal to 1 are deemed to be meaningful and are therefore retained, and those with eigenvalues less than 1.0 are considered to be “random noise”. This minimum numerical value not only removes the need for user interpretation of visual scree plots, but also means that the number of PCs used for each analysis can be automatically assigned through the use of this rule - resulting in greater efficiency and robustness, as well as a reduction in subjectivity and user input.

Therefore, for this research, the Kaiser Criterion was used to determine the number of PCs to be retained in each examination.

3.4.2.2 Linear Discriminant Analysis (LDA)

LDA is a supervised technique and has characteristics of Analysis of Variance (ANOVA), Multiple Linear Regression (MLR) and PCA. Supervised techniques are capable of predicting specific outcomes about data. To utilise supervised techniques, a subset of data points for which the outcomes are already known are required. This data is then used to "train" a model, allowing the machine to learn what a typical data point looks like for each of the outcomes. The model is then "tested" using new data for which the true outcome is unknown and makes a prediction as to the probable outcome given the training dataset.

As the outcome of the training dataset is known, the known outcomes are employed to derive linear combinations of variables called canonical variates (CVs) [67]. These CVs are constructed such that they maximise the separation (e.g. discrimination, classification) between known groups of samples [71] as opposed to the PC method which maximised the variation described. CVs are linear combinations of the original variables chosen in such a way that CV_1 reflects group difference as much as possible. CV_2 then captures as much as possible of the differences not displayed by CV_1 and so on. Each CV is a composite variable, made up of different contributions (donated by 'b' coefficients) of the x variables as shown in Equation 5. Wavelengths that can classify the samples more effectively will receive a higher importance, in terms of getting a larger coefficient value.

Equation 5: The General Equation for Canonical Variates

$$CV_1 = b1_1*x_1 + b1_2*x_2+\ldots\ldots b1_n*x_n$$

$$CV_2 = b2_1*x_1 + b2_2*x_2+\ldots\ldots b2_n*x_n$$

Using these CVs, LDA provides a probability of a fibre belonging to each one of the samples in question. These probabilities can then be used to propose if two samples are indistinguishable using our classification system.

LDA has been commonly used in previous studies where MVA has been applied to other evidence types. LDA uses a reduced dataset (utilising PCA or other means) to make a recommendation as to which outcome a sample is most likely to have. However, what makes the proposed application of LDA in this research novel, is that previous research often takes the recommendation as to which group a sample belongs to based purely on the highest probability value provided (i.e. largest number).. Furthermore, previous research often classifies samples into groups based on some previously constructed database, which may or may not be of a suitable size, robust and representative, rather than being able to make recommendations based on “live” data from the comparison being undertaken at that time. This research looks solely at the two groups of fibres and not a historical database and makes a recommendation as to whether they are indistinguishable or distinguishable – providing “sub-source” information which is vital to all further interpretation.

The recommendations made using LDA have similar desirable criteria as PCA, in that it utilise a probabilistic approach, requires minimal user input and it is robust (i.e. the same output should be given if the same data was analysed using the

same method by a different examiner) and was therefore deemed a suitable technique for investigation in the research.

In summary, this research focussed on two statistical approaches: the combined approach of PCA (for dimension reduction) followed by LDA and a direct application of LDA (with dimension reduction also being performed using LDA) – referred to as *PCA-LDA* and *LDA-own* respectively.

3.4.3 PCA-LDA

The *PCA-LDA* approach involves first reducing the wavelengths using PCA then subsequent classification using LDA, as the implementation of LDA requires more fibres than wavelengths before analysis can be performed. Objective classification functions are then constructed based upon using the reduced set of variables. PCA is used as a dimension reduction technique to take advantage of the fact that the absorbance values at across the wavelengths tend to be correlated. Typically, the first few PCs are sufficient to account for most of the variability in the dataset and are retained for further analyses, thus reducing the dimensionality of the dataset.

To determine the number of PCs to retain while still maintaining an objective and consistent approach, the Kaiser Criterion was used. Under the Kaiser Criterion, a PC is considered to be meaningful and hence retained if its eigenvalue is above 1 [143]. The eigenvalue measures a PC's ability to capture variability in the original data. The retained PCs are subsequently analysed using LDA.

3.4.4 LDA-own

As previously mentioned, the implementation of LDA requires more fibres than variables (e.g. wavelengths) before analysis can be performed in most widely available platforms e.g. SPSS. Therefore, in practice, dimension reduction techniques such as PCA are often used to reduce the dataset followed by LDA, which is then used to classify the data.

However, the *lda* function in R allows a direct analysis of the original data i.e. does not require more fibres than wavelengths, as dimension reduction is performed as part of the LDA process by the creation of CVs. The number of CVs created is a maximum of *n-1* where *n* is the number of samples in the dataset – ensuring that the number of samples is greater than the number of variables. This therefore potentially increases both the efficiency of analysis as well as avoiding the potential issue of useful information for discrimination being lost during the dimension reduction process [136].

3.5 Leave one out cross validation procedure

Leave one out cross validation (LOOCV) forms a part of the classification system proposed in this research, alongside the MVA method (either *PCA-LDA or LDA-own*), SPP, exceedance proportion and the three recommendation categories. Some previous applications of MVA to forensic evidence have used LOOCV to determine the classification accuracy of their models based on a small database created during their experiments. Although useful as a means of predicting the accuracy of a particular body of research, this may potentially lead to the same issue discussed in the National Research Council report [31] in which they caution against assigning numerical weight to results using a database that is not suitable in terms of size or representativeness. In this research, LOOCV is not used in the more traditional sense for method evaluation (i.e. to test the accuracy of a proposed system) but is used to ensure that each fibre is tested and contributes to the overall recommendation of the system.

Using 20 fibres in total as an example (e.g. fibres 1-10 in group 1, 11-20 in group 2), each fibre is left out in turn and the remaining fibres are used to “train” the system as to a typical dataset for groups 1 and 2. So in the first iteration, fibre 1 is excluded and the remaining 19 fibres (2-20) are used to train the model, with fibre 2-10 being group 1 and fibres 11-20 being group 2. Fibre 1 is “tested” against the two groups, its membership predicted and a SPP value obtained - the probability of assigning the held out fibre to its original group. This process is visualised in Figure 24.

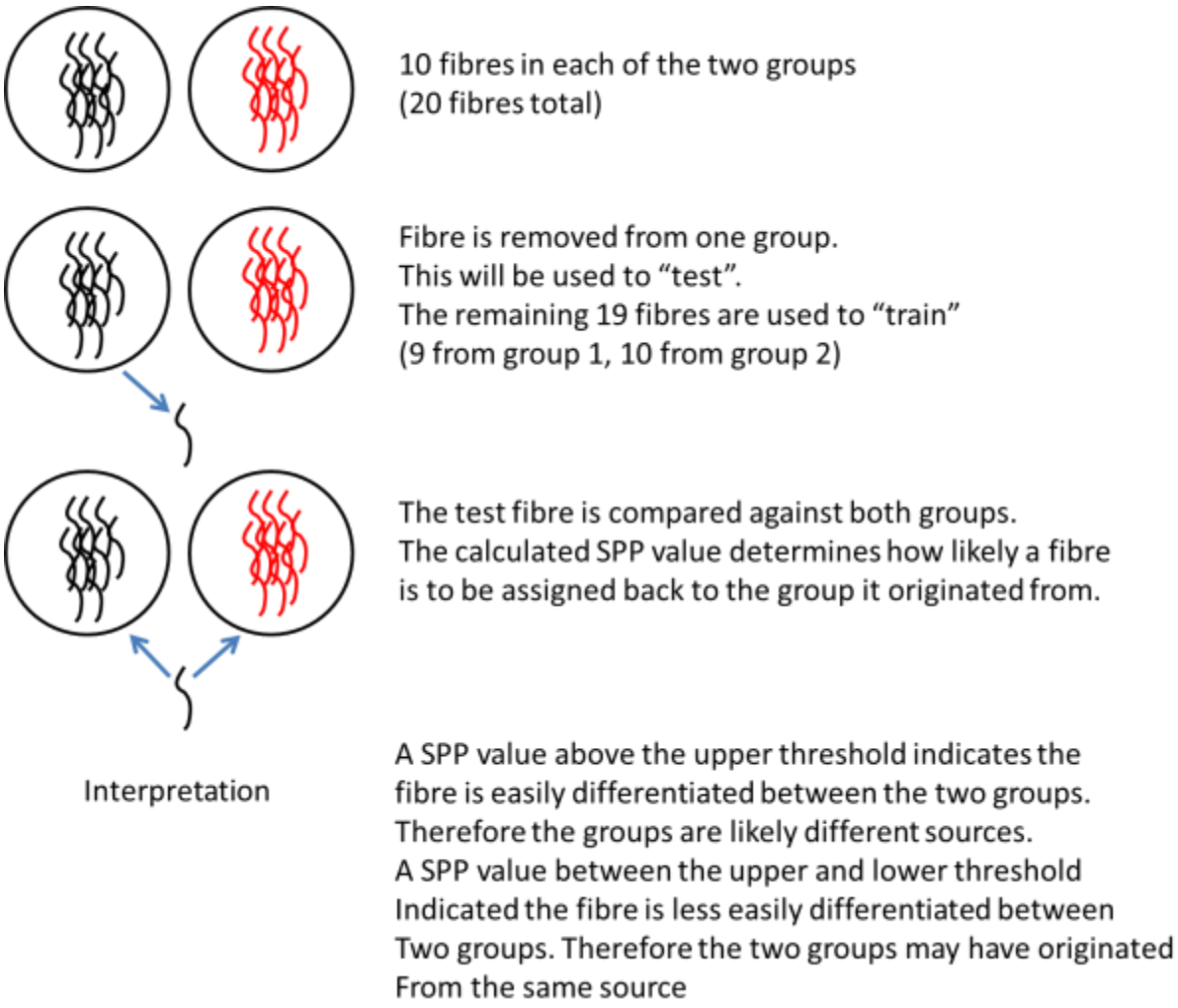

Figure 24: Visual representation of one iteration of leave one out cross validation

This process is then repeated by the system for each of the remaining fibres as shown in Table 7.

The total number of iterations performed depends on the total number of fibres being used. For example, if 20 fibres were used, this would be repeated 20 times – with each fibre being left out in turn, and the system "trained" using the data from the remaining fibres.

Table 7: All training and test groups when using LOOCV with 20 fibres total

	Group 1										Group 2									
Iteration	**Fibre 1**	**Fibre 2**	**Fibre 3**	**Fibre 4**	**Fibre 5**	**Fibre 6**	**Fibre 7**	**Fibre 8**	**Fibre 9**	**Fibre 10**	**Fibre 11**	**Fibre 12**	**Fibre 13**	**Fibre 14**	**Fibre 15**	**Fibre 16**	**Fibre 17**	**Fibre 18**	**Fibre 19**	**Fibre 20**
1	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
2	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
3	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
4	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
5	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
6	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
7	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
8	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
9	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
10	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train
11	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train	Train
12	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train	Train
13	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train	Train
14	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train	Train
15	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train	Train
16	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train	Train
17	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train	Train
18	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train	Train
19	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test	Train
20	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Train	Test

3.6 Self-predictive Probability (SPP)

Self-Predictive Probability (SPP) is defined as the probability of assigning a held-out fibre to its original group. So, for example, for a given fibre (which belongs to group 1) it is the probability of assigning that fibre to group 1. Similarly, if the held-out fibre is from group 2, then its SPP is defined as the probability of assigning that fibre to group 2. For each cross validated fibre, the (posterior) predictive probability of assigning the cross validated fibre to both of the samples presented in the training data was recorded by the system. These predictive probabilities were then used to evaluate the discriminating power and recommendation accuracy of the statistical methods in order to determine which was most suitable.

When the recovered fibre samples truly originated from different sources (and should be distinguishable), an ideal statistical method should be confident of assigning the individual fibres back to the true corresponding samples i.e. provide a high value for SPP as it is much easier to recommend being in its own group rather than an alternate group. If the samples originated from the same source (and should be indistinguishable), then the SPP should be lower as the statistical methods should be less confident in terms of assigning a fibre to one of the two groups, which would recommend the two groups to be indistinguishable. Various upper/lower SPP thresholds were investigated to determine the effect of these on classification accuracy.

Based on the resulting SPP value, each single cross-validated fibre is classified into the following categories in Table 8:

Table 8: Showing the relationship between SPP value and classification for the proposed classification system

Classification	SPP Value
Confident	SPP > upper threshold
Less Confident	lower ≤ SPP ≤ upper threshold
Potential misclassification	SPP < lower threshold

“Misclassification” in this context would suggest there may be an issue with the labelling of the fibre i.e. predicting which group it originated from as an SPP below the lower threshold means it is very dissimilar compared to the group it is trying to be put back into. This suggests a potential outlier, contamination or user error when labelling or inputting data.

3.7 Exceedance proportion

Across all recovered fibres, the proportion of fibres that fall into each of the three categories (confident, less confident and potential misclassification) is computed. Since LOOCV leaves each fibre out one at a time for testing and trains with the remaining fibres in the group, numerous classifications are made across a group of total fibres – each based on the SPP values obtained. For example, when 20 total fibres are used, 20 classifications are made (one for each fibre in terms of the SPP value obtained).

Therefore, to take into consideration all of the classifications across all fibres in both sets, and to obtain an overall recommendation, the use of an exceedance proportion was investigated. Various exceedance proportions were investigated to determine the required proportion of one particular recommendation (i.e. confident, less confident and potential misclassification) in order to be considered the overall recommendation – i.e. the final output.

The various exceedance proportions investigated were: 0.5, 0.6, 0.7, 0.8 and 0.9. For example, an exceedance proportion of 0.5 means that greater than 50% of the total recommendations to be in agreement (i.e. one of: confident, less confident and potential misclassification) in order for it to be the overall recommendation. It should be emphasised that this is **"greater than", not "greater than or equal to"** as the latter may result in a situation where 50% could be confident and 50% could be uncertain for example – resulting in conflicting recommendations. This is also why the values cannot reduce below 50%. In the case of 20 total fibres this would mean at least 11 of the 20 total recommendations must be in agreement in order to provide the overall recommendation.

The effect of altering the upper and lower SPP thresholds and the exceedance proportion was investigated to determine the optimal settings that resulted in the highest recommendation accuracy.

3.8 Summary

To make an overall recommendation, the following decision rules were proposed for the system:

- Two groups are considered to be “indistinguishable” from each other if the proportion of fibres with a less confident SPP classification is greater than the exceedance proportion.
- Two groups are considered to be “excluded” if the proportion of confident fibres based on the upper/lower SPP thresholds is greater than the exceedance proportion
- Otherwise, “no recommendation” will be provided.

4. Establishing the Optimal Settings for the Classification System

4.1 Introduction

The key innovation of this research is the proposed classification system that utilises a probabilistic, and therefore arguably more objective, approach to the evaluation of textile fibre evidence, in contrast to the current, arguably more subjective, methodologies [36,59]. Subjectivity, as is considered for the purposes of this research, relates to interpretation of forensic evidence that is opinion based – being influenced by an examiner's perception and/or their previous knowledge/experience [33,36,85].

4.1.1 The need for a model classification system

The National Research Council report [31] comments that the large amount of research into DNA has allowed for analysis to become "*less subjective*" and therefore more likely to be reliable. Subsequently, the intention of this classification system is to provide a robust and unbiased recommendation as to whether or not two groups of fibres are indistinguishable and may have therefore originated from the same source or distinguishable and therefore could not have originated from the same source. Objectivity can be increased with data collected by microspectrophotometry (MSP) and subsequent analysis using multivariate analysis (MVA) and machine learning.

4.2 Multivariate Analysis (MVA) in Forensic Science

MVA is a class of statistical techniques used to examine relationships within large complex datasets and has previously been applied in the analysis of forensic samples including paint [70,81–83,116], inks [76–80,114,115], hair [139] and drugs [112,144–147]. Recently, MVA has also seen increased application to the analysis of textile fibres [67,71,136,137,148]. However, these previous fibre studies utilising MVA do not directly address the fundamental question in the evaluation of fibre evidence: are the compared groups indistinguishable and therefore potentially originate from the same source in a definitive way? By looking at the sub-source indistinguishable or distinguishable question, this research shows a greater applicability to forensic casework and the potential application to future studies.

Many of the previous studies involving the use of MVA in a forensic science context have utilised linear discriminant analysis (LDA) [67,71,79,115,139,148–150] and principal component analysis (PCA) [67,71,79,81,112,115,116,136,139,146,148,149,151] either in conjunction (e.g. PCA as a dimension reduction technique or a classification technique followed by LDA) or as standalone techniques. PCA-LDA and LDA are standard, accepted and well-known MVA methods that have seen application in other forensic research, and as such have been used as the starting point in this research.

4.2.1.1 Principal Component Analysis (PCA)

PCA is an unsupervised technique [152]. Unsupervised techniques do not focus on predetermined attributes, nor do they predict a target value. Rather, unsupervised techniques find hidden structure and relationships amongst the data.

PCA is however also commonly used as a dimension reduction technique prior to other analysis such as LDA. PCA as a dimension reduction tool is a technique that reduces the dimensionality of the dataset by finding a smaller number of latent variables that explain the ***maximum variance*** by constructing linear combinations of the original variables. These are called principal components (PC) [141].

In the example of this research, the utilisation of PCA as a dimension reduction technique can reduce the number of variables from 404 (the number of wavelengths examined when utilising the visible range (380 nm – 710 nm), to less than 10 PCs.

The use of PCA for dimension reduction is considered suitable for this research which aims to provide a more objective method, requiring minimal user input as the process of determining which data to retain. This is based on mathematical calculations when utilising the Kaiser Criterion (whereby only PCs with eigenvalues greater than 1 are retained [143]). The eigenvalue measures the ability of a PC to capture variability in the original data. Therefore, determining the number of PCs to retain would be consistent if anyone were to perform the same dimension reduction using PCA on the same dataset - making it more robust and reproducible. This may not be the case if utilising visual interpretation of scree plots as these require subjective interpretation and may not always be straight forward to interpret potentially leading to disagreements between examiners as to how many PCs to retain for further analysis. This in turn could result in different datasets being used, and subsequently different decisions being made.

4.2.1.2 Linear Discriminant Analysis (LDA)

Unlike PCA, LDA is a supervised technique [57,82]. As such, it utilises both group membership and a set of predictor variables to construct classification functions. Specifically in this research, the predictor variables are the absorbance values obtained across all of the 404 wavelengths in the visible range MSP spectra (380 – 710 nm) and the group membership indicates which sample each fibre belongs to e.g. “lightberry”. This means that the known group membership of all spectra is employed to derive linear combinations of variables called canonical variates (CVs) [67]. These CVs are constructed such that they maximise the separation between known classes of samples [71] meaning LDA creates a model that maximises discrimination between the assigned groups in the original data, and can be used to predict the classification of new samples using the established model. This differs from the approach of PCA, which maximises variation - the two are not necessarily the same.

The application of LDA requires more samples than variables [140] - which is why it is often performed after using a dimension reduction technique such as PCA. However, when using the *lda* function in R prior dimension reduction is not necessary. n-1 CVs (where n = number of samples) are created if the number of samples is greater than number of variables, resulting in a new dataset from the data which satisfies the need for more samples than variables.

Using these CVs, R was programmed to calculate a probability of a fibre belonging to each one of the samples in question; calculating the probability of assignment back to its own group for each fibre - the self-predictive probability (SPP). The concept of SPP is described in more detail in the previous chapter - but briefly, it is the probability of a fibre assigning back to its “own” group, rather than the other

group. This can then be combined with exceedance proportion (E.P.), which determines the proportion of classifications that must be in agreement in order to be the final recommendation. These probabilities can then be used to propose if two samples are indistinguishable or distinguishable by using the calculated probabilities alongside proposed decision boundaries (upper/lower SPP) to determine if the samples are recommended to be “indistinguishable”, “excluded” or if “no recommendation” can be given.

This SPP approach allows for movement away from the more subjective element involved with classification based on opinion and experience (the human factor [32,153]) and towards a more robust and objective approach reliant on probabilistic approaches.

LDA as a standalone technique, as well as PCA followed by LDA, were investigated in this research as a means to determine a probability on which a indistinguishable/distinguishable decision can be made as these not only represented two of the most common methodology encountered in the literature but LDA also provides one of the most objective methods of analysis. These two statistical approaches: (a) the combined approach of principal component analysis (PCA) with linear discriminant analysis (LDA) and (b) a direct application of LDA; are labelled as PCA-LDA and LDA-own, respectively.

4.2.2 Previous studies utilising MVA for textile fibres

4.2.2.1 Deviterne-Lapeyre, Buzzini and Massonnet

Deviterne-Lapeyre, Buzzini and Massonnet [136] studied 20 blue acrylic samples - comprising 60 fibres in total. They obtained three spectra from each fibre, and

used two of the three spectra plus the average of all three spectra to comprise their dataset for each fibre. The two of three spectra to be utilised was determined by human evaluation as to which two were most similar. The authors used PCA as a classification technique to attempt to differentiate each of 20 blue acrylic sources. The authors state they were able to separate 18 of 20 fibres into different groups - although the interpretation of the data required a large amount of subjective, opinion based interpretation to come to this conclusion.

In the view of this research, the human evaluation of clusters and grouping utilising PCA introduces too much subjectivity to the analysis to be used as a suitable model to follow. Therefore PCA as a classification method is not considered in this research.

Deviterne-Lapeyre, Buzzini and Massonnet also performed hierarchical clustering analysis (HCA); a common MVA technique often used in the analysis of a variety of forensic evidence types which aims to group samples based on their levels of dissimilarity [81,82,116,138]. However, similarly to using PCA as a classification tool, it is thought that the human evaluation of decision boundaries would again introduce subjectivity into the analysis. In addition, the distance of the decision boundaries is unlikely to remain constant and would need to be altered for each new analysis – reducing the reliability of the approach by not providing a robust set of criteria that can be used across multiple scenarios. Therefore HCA was not considered for this research.

4.2.2.2 Bianchi, Riboni, Trolla, Furlan, Avantaggiato, Iacobellis and Careri.

Bianchi *et al.* [148] used Raman spectroscopy followed by PCA and LDA to classify cotton fibres. The authors used data in the study that was pre-processed using a *"Savitzky-Golay filter using a five-point smoothing window and a second order polynomial deconvolution followed by standard normal variate algorithm"* [148]. This pre-processing is an area where this research project differs, as by not requiring pre-processing before analysis, the amount of user input has been reduced in order to contribute towards the desirable criteria outlined earlier in this chapter as well as in the previous chapters of minimising user input - as well as small reductions in time efficiency and transparency of analysis by reducing the number of button clicks and processing time.

Bianchi *et al.* used leave one out cross validation (LOOCV) in a more traditional application - to estimate the accuracy and predictive ability of their model based on their training datasets. Up to 100% classification accuracy was reported by *Bianchi et al.* for three out of four of the series under investigation utilising LOOCV; however some situations resulted in classification accuracy as low as 67% when LOOCV was performed. This could suggest that their datasets may be less useful as a predictive tool going forward when working with more casework like samples which would likely include more variation and be more challenging than those used in their study.

As discussed previously, in this research LOOCV is used as part of the classification system as opposed to as a measure of the accuracy of the model. By utilising this more novel approach, it is ensured that all iterations of fibre groupings are used when making the final decision avoiding an over reliance on the outcome of one grouping which may or may not have been swayed to one extreme or the

other. This therefore considers intra-sample variation much more than previous studies appear to. Therefore, this not only demonstrates a novel application of this research, but also provides a more comprehensive and considered dataset on which to base any decision.

4.3 Experimental Design Rationale

Given the methodology and application proposed, this chapter considers a set of scenarios to determine accuracy of the proposed classification system in “straight forward” situations (whereby the visual and spectral features of each fibre are obviously indistinguishable or distinguishable) before more complicated, casework like, scenarios are examined in subsequent chapters.

This evaluation of accuracy was achieved by analysing sample sets of fibres larger than those utilised in any published studies previously [67,71,136,148,154] allowing for more comprehensive datasets and therefore more robust and meaningful evaluation of the success of the system, while still remaining true to real life questions (i.e. the indistinguishable or distinguishable question) to maximise the potential for future application to forensic casework. It also looks to implement a more definitive and probabilistic based approach.. Further to this, an attempt is made to identify and investigate any potential limitations of the proposed classification system at this early stage in order to satisfy the desirable criteria of reports such as the National Research Council and other bodies such as the President’s Council of Advisors on Science and Technology (PCAST) [31,155] who have previously published concern with the lack of reporting of limitations.

4.4 The Classification System

Given two groups of fibres, the classification system utilises MSP data to decide whether the two groups of fibre dyes are indistinguishable and therefore may have originated from the same source or distinguishable and therefore originate from different sources - as would be done manually in any fibre comparison process. Therefore this step is being replicated, but in an arguably more objective manner. If neither of these conditions can be satisfied based on the proposed criteria, the system is unable to confidently decide on one outcome or another then “no recommendation” is given as an output.

This chapter builds on the information and rationale provided in the previous chapters and evaluates a set of experiments to contribute to the determination of a set of “optimal settings” to fulfil the requirements of a model classification system - assessed by considering the mean correct recommendation accuracy i.e. where two groups of fibres are decided to be indistinguishable when they truly originate from the same source, and distinguishable when they truly originate from different sources. These optimal settings will then be used for research and experiments going forward that investigate more complicated and casework-like situations such as blocks of colour (where the groups of fibres being examined are of the same fibre type and broad colour), single fibre situations (where only a single questioned fibre is available, therefore potentially causing issues with determining a representative sample [33,85]) and also when investigating the limits of detection of the classification system utilising own dyed fibres.

4.5 Aims and Objectives

To date, limited published material is available regarding the use of MVA with application to textile fibre evidence. The aim of this chapter of the thesis is to establish and make clear a set of optimal settings to be used going forward by combining objectives 1-4 below.

The key objectives which are investigated in this chapter are to assess the following, and their effect on classification accuracy:

1. The proposed MVA methods: PCA-LDA and LDA-own
2. The optimal self-predictive probability (SPP)
3. The optimal exceedance proportion (E.P.)
4. The number of fibres to be used

4.6 Methodology

4.6.1 Fibre Type Selection

PCA-LDA and LDA-own approaches were evaluated using cotton fibres as these represent the most common natural fibre type encountered in fibre population studies and in forensic casework [5,7–9,11,21,156–158]. Additionally, acrylic was investigated as an example of a synthetic fibre. Although relatively common, other synthetic fibres such as polyester have become mpre common recently and polyester has seen “an extensive increase in production” [16]. A selection of fibre population studies, the year they were performed, and the most common fibre type/colour combinations recorded are listed in Table 9 – although it has been noted by Robertson *et al.* that in many cases colour is assessed subjectively and without the aid of MSP, but that even without measurement by MSP the chance of one type of synthetic fibre constituting >1% of a random population was “very small” [4]. The fibres selected here are visually and spectrally distinguishable (i.e. should pose no difficulties to a fibre examiner or an ideal classification system) – as if it does not prove to be successful with these in simple situations it is unlikely to be suitable for real forensic casework.

Table 9: The results of various previous fibre population studies (adapted from Palmer [38]**)**

Authors (Year)	Substrate	Abundant Fibre Colour/Type
Grieve & Biermann (1997a)	Outdoor surfaces	Grey-black Cotton (23.8%) Blue Cotton (13.3%)
Roux & Margot (1997a)	Car seats	Grey-black Cotton (17.3%) Blue Cotton (16.4%)
Massonnet *et al* .(1998)	T-shirts	Grey-black Cotton (24%) Blue Cotton (14%)
Cantrell *et al.* (2001)	Cinema seats	Grey-black Cotton (33.4%) Blue Cotton (29.6%)
Palmer & Oliver (2004)	Head hair	Grey-black Cotton (26%) Blue Cotton (23%)
Watt *et al.* (2005)	Washing machines	Black cotton (26.9) Blue cotton (20.2%)
Was-Gubala (2009b)	Public transport	Grey-black Cotton (25%) Blue Cotton (15%)
Palmer & Burch (2009)	Human skin	Grey-black Cotton (37%) Blue Cotton (17%)
Lazic *et al.* (2012)	Cinema seats	Black cotton (46%) Blue cotton (20%)

4.6.2 Mounting fibres and obtaining MSP data

For both acrylic and cotton sources, fibres were scraped from the surface of a fibre shade card and mounted on glass slides in phytohistol. MSP was then performed in the VIS range (380 – 710 nm), taking readings from 40 fibres from each source. Each of these 40 fibres had three individual readings taken along their length that were averaged to produce one spectrum per fibre [41,136]. An average spectrum was used to minimise the effect of intra sample variation caused by variation in dye uptake along the length of the fibre – something that is more common in

natural fibres, such as cotton [3,29,58,85]. The 40 spectra for each source were then subdivided into smaller groups as required e.g. into four groups of 10 or two groups of 20.

4.6.3 Multivariate analysis interpretation of MSP data

Datasets were interpreted using the proposed R based model classification system, using the combinations of self-predictive probability (SPP) and Exceedance Proportion (E.P.) thresholds shown in Table 10 to determine which SPP and E.P. combination that provided the highest classification accuracy. These SPP thresholds were established by evaluation of some preliminary data to determine the range of SPP values obtained from trial datasets. The E.P. values represent proportions of a dataset (e.g. 0.5 = 50%, 0.9 = 90%). No values used 0.5 for E.P. were considered as to retain at least a majority outcome, but higher proportions were considered to see if a larger majority resulted in improved accuracy.

Table 10: The Upper/Lower SPP Thresholds and Exceedance Proportions Investigated

Upper/Lower SPP Thresholds	Exceedance Proportion (E.P.)
0.9999/0.0001	0.5
0.999/0.001	0.6
0.99/0.01	0.7
0.95/0.05	0.8
	0.9

4.6.4 “Single Source” and “Pairwise” Scenarios

The objective is for the classification system to correctly classify “indistinguishable” and “distinguishable” fibres - akin to the decision of a fibre examiner. So, two experimental set-ups were created to test both situations; “single source” and “pairwise”. The “single source” scenario compared groups of fibres that originated from the same source. The correct recommendation in this situation would be “indistinguishable” since the fibres originate from the same source. On the other hand, the “pairwise” scenario compared groups of fibres that originated from distinguishable sources and should therefore be recommended to be “excluded” since these fibres originate from different sources. In the single source scenario, each subdivided (e.g. groups of 10 from 40) pool of fibres from each source was split into two equally sized groups 50 times with each dividing process containing a different combination of fibres in each group.

This splitting of the dataset into 50 different groupings was performed to avoid an over-interpretation from a single grouping of fibres – resulting in more robust data and evaluation of the outcome.

The pairwise scenario **c**onsists of an exhaustive set of two-sample combinations from different sources. In the pairwise scenario, for five fibres per group (10 fibres total), five fibres from each sample of fibres was compared against five fibres from each other sample of fibres until all possible pair combinations were exhausted. Similarly for 10 fibres per group (20 fibres total) 10 fibres from each sample of fibres was compared against 10 fibres from each other sample of fibres until all possible pair combinations were exhausted. Using 23 different sources (as used for both acrylic and cotton) resulted in 253 possible unique pairwise combinations for each fibre type.

The full list of pairwise comparisons involving two samples at a time (numbered 1-23 to save space and be interchangeable between the two fibre types) is demonstrated in Table 11 - where the greyed out area represents either a comparison of the group with the same source (as in the single source setting) or a pairwise comparison that has already been performed e.g. when using acrylic sources, grenadine against light berry provides the same comparison and results as light berry against grenadine.

Table 11: Demonstrating the 253 possible different pairwise combinations when using 23 different sources

Source	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1		1v2	1v3	1v4	1v5	1v6	1v7	1v8	1v9	1v10	1v11	1v12	1v13	1v14	1v15	1v16	1v17	1v18	1v19	1v20	1v21	1v22	1v23
2			2v3	2v4	2v5	2v6	2v7	2v8	2v9	2v10	2v11	2v12	2v13	2v14	2v15	2v16	2v17	2v18	2v19	2v20	2v21	2v22	2v23
3				3v4	3v5	3v6	3v7	3v8	3v9	3v10	3v11	3v12	3v13	3v14	3v15	3v16	3v17	3v18	3v19	3v20	3v21	3v22	3v23
4					4v5	4v6	4v7	4v8	4v9	4v10	4v11	4v12	4v13	4v14	4v15	4v16	4v17	4v18	4v19	4v20	4v21	4v22	4v23
5						5v6	5v7	5v8	5v9	5v10	5v11	5v12	5v13	5v14	5v15	5v16	5v17	5v18	5v19	5v20	5v21	5v22	5v23
6							6v7	6v8	6v9	6v10	6v11	6v12	6v13	6v14	6v15	6v16	6v17	6v18	6v19	6v20	6v21	6v22	6v23
7								7v8	7v9	7v10	7v11	7v12	7v13	7v14	7v15	7v16	7v17	7v18	7v19	7v20	7v21	7v22	7v23
8									8v9	8v10	8v11	8v12	8v13	8v14	8v15	8v16	8v17	8v18	8v19	8v20	8v21	8v22	8v23
9										9v10	9v11	9v12	9v13	9v14	9v15	9v16	9v17	9v18	9v19	9v20	9v91	9v22	9v23
10											10v11	10v12	10v13	10v14	10v15	10v16	10v17	10v18	10v19	10v20	10v21	10v22	10v23
11												11v12	11v13	11v14	11v15	11v16	11v17	11v18	11v19	11v20	11v21	11v22	11v23
12													12v13	12v14	12v15	12v16	12v17	12v18	12v19	12v20	12v21	12v22	12v23
13														13v14	13v15	13v16	13v17	13v18	13v19	13v20	13v21	13v22	13v23
14															14v15	14v16	14v17	14v18	14v19	14v20	14v21	14v22	14v23
15																15v16	15v17	15v18	15v19	15v20	15v21	15v22	15v23
16																	16v17	16v18	16v19	16v20	16v21	16v22	16v23
17																		17v18	17v19	17v20	17v21	17v22	17v23
18																			18v19	18v20	18v21	18v22	18v23
19																				19v20	19v21	19v22	19v23
20																					20v21	20v22	20v23
21																						21v22	22v23
22																							22v23
23																							

4.7 Results & Discussion

It was expected that a suitable classification system would show high accuracy when making recommendations that two groups of fibres that originated from the same source were “indistinguishable”, and two groups of fibres that originated from visually and spectrally distinguishable sources would be being “excluded”. Each MVA, SPP, E.P. and number of fibres per group combination was assessed by determining the number of correct recommendations and dividing by the total number or recommendations. These results were then expressed as a percentage.

To be deemed successful, an arbitrary threshold of 90% accuracy was required for the setting combination to be considered for future studies and applications in the first instance. This 90% threshold allowed for some simple exclusion of unsuccessful techniques in order to allow further focus on those with higher potential.

4.7.1 Single Source Setting – Five Fibres Per group

The “single source” scenario was investigated first, whereby the analysed fibres originated from the same source. Classification accuracy was determined by dividing the number of “indistinguishable” (since the two groups of fibres truly originated from the same source) recommendations by the total number of comparisons performed. Both the PCA-LDA and LDA-own approaches were investigated using both acrylic and cotton fibre samples.

The results from this first experiment are shown in Figure 25.

Heat Map Scale: 100% 90% 80% 70% 60% 50% 40% 30% 20% 10% 0%

Acrylic – PCA-LDA method – five fibres per group

Upper SPP \ E.P.	0.5	0.6	0.7	0.8	0.9
0.9999	98.8%	98.1%	96.8%	90.0%	68.0%
0.999	98.3%	96.8%	93.7%	83.2%	55.6%
0.99	95.6%	92.1%	85.1%	66.5%	35.4%
0.95	89.2%	82.3%	67.4%	43.9%	16.3%

Cotton – PCA-LDA method – five fibres per group

Upper SPP \ E.P.	0.5	0.6	0.7	0.8	0.9
0.9999	99.3%	98.6%	97.1%	90.0%	64.8%
0.999	98.2%	97.1%	92.8%	82.1%	50.2%
0.99	95.5%	91.1%	83.6%	65.9%	31.6%
0.95	87.3%	81.6%	64.5%	40.8%	13.0%

Acrylic – LDA-own method – five fibres per group

Upper SPP \ E.P.	0.5	0.6	0.7	0.8	0.9
0.9999	74.3%	59.2%	39.5%	18.8%	5.1%
0.999	58.9%	41.5%	23.8%	8.3%	2.2%
0.99	35.1%	18.6%	7.3%	2.0%	0.4%
0.95	14.0%	4.0%	0.6%	0.2%	0.0%

Cotton – LDA-own method – five fibres per group

Upper SPP \ E.P.	0.5	0.6	0.7	0.8	0.9
0.9999	77.4%	63.0%	46.8%	24.9%	6.4%
0.999	64.6%	47.2%	29.7%	13.0%	2.2%
0.99	41.2%	23.8%	10.5%	2.8%	0.1%
0.95	16.2%	6.1%	1.4%	0.2%	0.0%

Figure 25: The effect of various exceedance proportion (E.P.) and self-predictive probability (SPP) value combinations on the mean correct recommendation rate of acrylic (left column) and cotton fibres (right column) when using five fibres per group and PCA-LDA (top row) or LDA-own (bottom row) methods

From the above results it can be observed that when using five fibres per groups, regardless of whether using the PCA-LDA or LDA-own approach, the highest classification accuracy was observed when using an upper/lower SPP threshold of 0.9999/0.0001 and E.P. of 0.5. The classification accuracy tended to decrease as the exceedance proportion increased (e.g. from 0.5 to 0.6), and/or the upper SPP decreased e.g. from 0.9999 to 0.999).

As the exceedance proportion increases, the likelihood of a “no recommendation” outcome being given increases as it becomes harder for the required proportion of recommendations to be consistent – i.e. if using 0.7 as the exceedance proportion then at least 7 of 10 recommendations must be consistent or “no recommendation” will be given – up from the at least 5 of 10 when using a 0.5 exceedance proportion.

As the upper SPP decreases, it becomes easier for the system to probabilistically recommend that a fibre is more likely to be assigned back to its “self” group, rather than the other group – meaning it is easier for an “excluded” recommendation to be obtained. Therefore, it is logical that this higher SPP value would suit the “single source” scenario best as it considers fibres from the same source – meaning that these fibres should be indistinguishable and having a lower SPP value may increase the number of false exclusions (i.e. “excluded” recommendations).

Both cotton and acrylic fibres showed a high classification accuracy (~99% accuracy) when using the PCA-LDA approach. This is likely because PCA is being used as a dimension reduction technique prior to LDA. When PCA is used for dimension reduction, PCs are constructed in such a way that the first PC accounts

for the most variation in the dataset – meaning that similar groups should be accounted for by similar PCs.

However, when using LDA-own, the classification accuracy drops to ~74% for acrylic fibres, and ~77% for cotton fibres. This is because the dataset is being reduced in a different way (using canonical variates (CVs) rather than PCs) which maximises differences in the dataset rather than extenuating similarities. Since the "single source" scenario is designed such that fibres originate from the same source, there should be very minor difference between the fibres, meaning that any differences highlighted could be the result of variation in the sample set. Since only five fibres have been used per groups here, it is possible that not enough fibres have been included to create a truly representative sample which may have allowed for the successful application of LDA-own – resulting in a high false exclusion rate.

4.7.2 Pairwise Setting – Five fibres per group

The optimal results from the previous experiment (fibres per group = 5, upper SPP = 0.9999, E.P. = 0.5, PCA-LDA approach) were then tested in the "pairwise" scenario. These optimal settings from the same source scenario were used for this next stage of the experiment as an ideal classification system should be accurate at making recommendations regardless of it the fibres could have originated from the same source or are from different sources.

Classification accuracy was determined by dividing the number of "excluded" (as the two groups of fibres truly originated from different sources) recommendations by the total number of comparisons – with the results being shown in Figure 26.

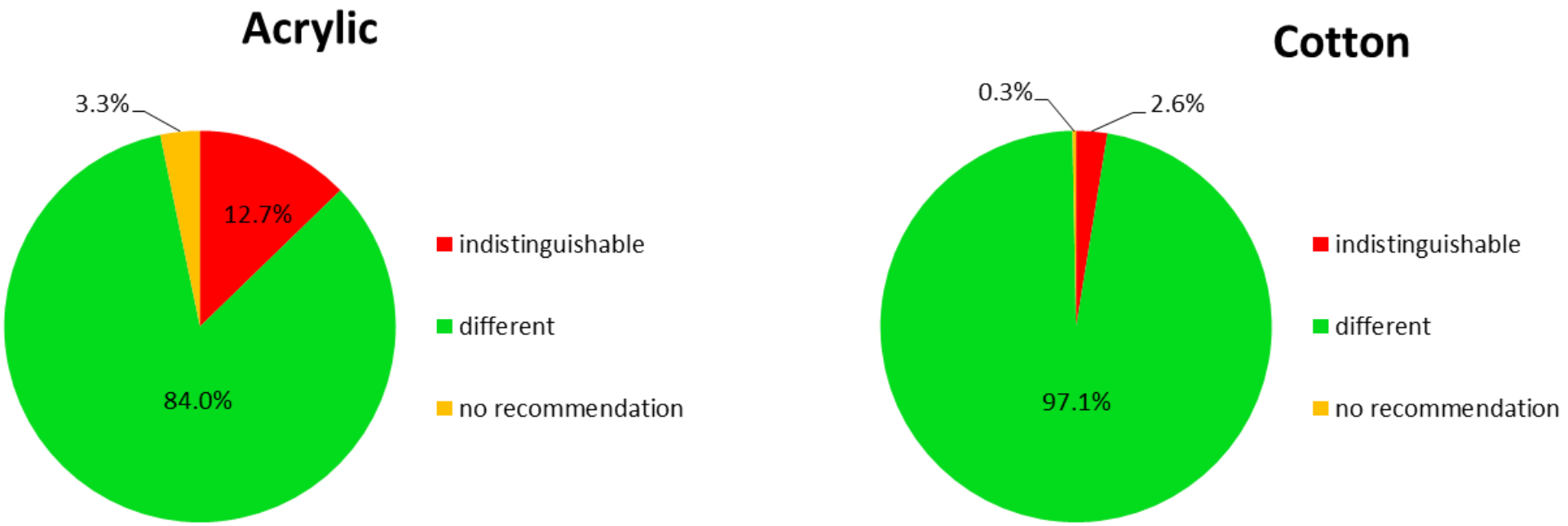

Figure 26: The percentages of correct (“excluded”) and incorrect ("indistinguishable" or "no recommendation”) recommendations when using the previously identified optimal settings (SPP = 0.9999/0.0001, E.P. = 0.5, PCA-LDA method) with acrylic and cotton fibres (five fibres per group)

From the above results it can be observed that when using five fibres per groups, upper SPP = 0.9999, E.P = 0.5 and the PCA-LDA approach, that high classification accuracy (~97%) was observed when interpreting cotton fibres, but less so when interpreting acrylic fibres (~84%). This could be a result of similar reasons discussed above, whereby the PCA dimension reduction is looking to describe variation in the dataset – not maximise the differences between the datasets. Therefore the reduced dataset is perhaps not as suitable to successfully differentiate two groups of fibres. This issue could be further amplified by the nature of acrylic fibre dyes compared to cotton fibre dyes demonstrating less variation due to dye uptake [3,29,58,85], meaning that less information is likely to be available within the spectra to allow for successful discrimination – as well as sample sizes.

With regards to sample size, the European Textile and Hair Group Guidelines [41] recommend that naturally occurring fibres such as cotton have at least ten fibres examined from each source where possible to ensure that this intra-sample variation is properly captured before interpretation. However, utilising five fibres per group was trialled first as the fewer fibres required the better due to the principles of fibre persistence [131]. A similar approach utilising 10 fibres per group could therefore be beneficial to the proposed MVA approach to ensure that the system has the adequate data available to make accurate and robust recommendations.

At this stage, no one set of optimal settings was established, as the PCA-LDA with five fibres per group did not offer sufficient accuracy for both the “single source” and “pairwise” scenario – regardless of the SPP and E.P. combination. Therefore, these experiments were repeated, but the number of fibre per groups was

increased to ten to see if capturing larger amounts of data prior to MVA resulted in greater accuracy.

4.7.3 Single Source Setting – Ten Fibres Per group

The results of the “single source” experiments, using ten fibres per group, are shown in Figure 27.

Heat Map Scale:	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	0%

Acrylic – PCA-LDA method – ten fibres per group

Upper SPP \ E.P.	0.5	0.6	0.7	0.8	0.9
0.9999	99.9%	99.9%	99.9%	99.9%	99.8%
0.999	99.9%	99.9%	99.8%	99.8%	99.6%
0.99	99.8%	99.8%	99.6%	99.3%	96.3%
0.95	99.6%	99.3%	98.6%	95.7%	82.5%

Cotton – PCA-LDA method – ten fibres per group

Upper SPP \ E.P.	0.5	0.6	0.7	0.8	0.9
0.9999	100.0%	100.0%	100.0%	100.0%	99.9%
0.999	100.0%	100.0%	100.0%	100.0%	99.3%
0.99	100.0%	100.0%	99.9%	99.7%	95.3%
0.95	99.7%	99.3%	98.4%	94.9%	81.1%

Acrylic – LDA-own method – ten fibres per group

Upper SPP \ E.P.	0.5	0.6	0.7	0.8	0.9
0.9999	95.3%	87.9%	73.7%	49.3%	16.1%
0.999	86.9%	78.4%	59.3%	37.7%	11.4%
0.99	56.4%	33.3%	12.4%	2.9%	0.2%
0.95	19.4%	21.2%	8.3%	2.1%	0.2%

Cotton – LDA-own method – ten fibres per group

Upper SPP \ E.P.	0.5	0.6	0.7	0.8	0.9
0.9999	96.0%	92.0%	83.7%	65.0%	31.4%
0.999	90.7%	81.7%	65.5%	41.5%	12.0%
0.99	71.3%	50.9%	28.9%	10.7%	1.7%
0.95	32.6%	13.3%	5.3%	1.3%	0.0%

Figure 27: The effect of various exceedance proportion (E.P.) and self-predictive probability (SPP) value combinations on the mean correct recommendation rate of acrylic (left column) and cotton fibres (right column) when using ten fibres per group and PCA-LDA (top row) or LDA-own (bottom row) methods

From the above results, when number of fibres is increased from five to ten, the same general trend is observed in that when the upper SPP is decreased from 0.9999 to 0.999, 0.99 or 0.95 and when E.P. is increased from 0.5 to 0.6, 0.7, 0.8 or 0.9 mean correct recommendation percentage decreases. However, different from when using five fibres per groups, a high classification accuracy is observed for both fibre types when using PCA-LDA (~100%) and LDA-own (>95%) approaches.

4.7.4 Pairwise Setting – Ten fibres per group

From the results above, both PCA-LDA and LDA-own are now suitable for further testing using the "pairwise" setting with upper SPP = 0.9999, E.P = 0.5, number of fibres per group = 10 to determine if optimal settings can be established to use for both scenarios. The results of these experiments are shown in Figure 28.

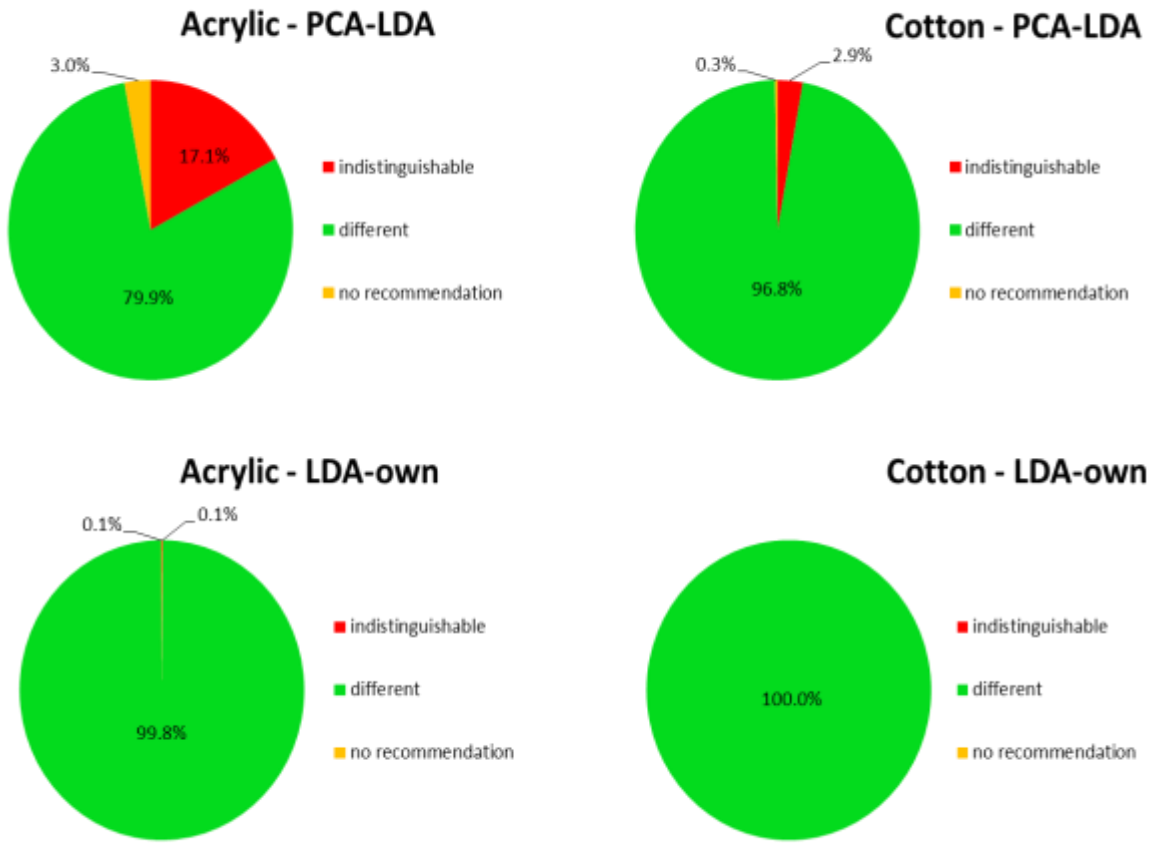

Figure 28: The percentages of correct (“excluded”) and incorrect ("indistinguishable" or "no recommendation”) recommendations when using the previously identified optimal settings (SPP = 0.9999/0.0001, E.P. = 0.5, ten fibres per group) with acrylic (left side) and cotton (right side) fibres for both PCA-LDA (top row) and LDA-own (bottom row).

From the results above, it can be seen that although the PCA-LDA approach performed well in the “single source” scenario, it does not have high classification accuracy (~80%) when working with acrylic fibres when using ten fibres per group with the optimal upper SPP and E.P. However, LDA-own shows high classification accuracy (~100%) for both acrylic and cotton fibre types.

Deviterne-Lapeyre, Buzzini and Massonnet [136] studied 20 blue dyed acrylic samples.

The authors state they were able to separate 18 of 20 fibre dyes into different groups (90% accuracy) - although the interpretation of the data required a large amount of subjective, opinion based interpretation to come to this conclusion. The results presented in Figure 28 show that when using LDA-own with acrylic fibres, 99.8% accuracy was observed and when using PCA-LDA 79.9% accuracy was observed. These numbers were 100% and 96.8% when using LDA-own and PCA-LDA respectively with cotton fibres. These demonstrate that in three of four combinations, a higher classification accuracy was observed than that by Deviterne-Lapeyre, Buzzini and Massonnet while utilising a more objective system and considering a larger variety of fibres types and colours than this previous study.

Bianchi *et al.* [148] used Raman spectroscopy followed by PCA and LDA to classify cotton fibre dyes. The authors used data in the study that was pre-processed using a *"Savitzky-Golay filter using a five-point smoothing window and a second order polynomial deconvolution followed by standard normal variate algorithm"* [148]. Up to 100% classification accuracy was reported by *Bianchi et al.* for three out of four of the series under investigation utilising LOOCV; however some situations resulted in classification accuracy as low as 67% when LOOCV was performed. As above, the classification accuracy observed to date in this research also showed three of four outcomes having high accuracy (above 96.8%) and the final outcome being 79.9%. Therefore, this would suggest that the research in this thesis provides a more reliable classification system, where even in the lowest output, a higher classification accuracy is observed. It is however

worth noting that the two studies utilise different applications of LOOCV so the results may vary if the same applications were used to assess.

The previous chapter considered a set of experiments wherein extensive testing was performed to determine the optimal settings for the proposed model classification system. Cotton and acrylic fibres were examined as these represent two of the most common natural and synthetic fibre types, respectively, encountered in fibre population studies [6–9,11,15,21,23,24]. Two scenarios were considered: where two groups of fibres originated from the same source and should therefore be recommended to be “indistinguishable”, and where two groups of fibres originated from different sources and should therefore be recommended to be “excluded” by the model classification system.

Two different multivariate analysis (MVA) / machine learning approaches were considered in the above scenarios, and their accuracy was assessed based on the percentage of correct recommendations made. These two approaches were:

- The application of principal component analysis (PCA) as a dimension reduction technique, followed by linear discriminant analysis (LDA) – **PCA-LDA**
- The application of LDA solely - utilised for both dimension reduction and classification – **LDA-own**

Regardless of which MVA approach was utilised, the highest mean correct recommendation percentage was obtained utilising the following settings:

- **Upper/lower self-predicative probability (SPP)** = **0.9999/0.0001**
- **Exceedance proportion (E.P.) = 0.5**
- **Number of fibres per group = 10**

4.7.5 Chapter rationale

However, the previous chapter was only the beginning of the required work in order to help establish a reliable and robust classification system. Fibre dyes that were often obviously visually and spectrally distinguishable when originating from different sources – and therefore as well as being correctly recommended by the classification system, should have also been correctly recommended by a suitably trained fibre examiner. Similarly, when originating from the same sources, fibres were scraped and mounted on the same slide before microspectrophotometry (MSP) readings were taken and the groups of fibres subdivided to create larger datasets and more robust evaluation of the outcomes.

To complete the validation studies and establish the applications and limitations of the classification system, datasets more akin to that given to fibre examiners were required to help establish the feasibility and reliability of the classification system. Therefore, this chapter looks at making comparisons between groups of fibres of the same broad colour and fibre type, e.g. red cotton, and assessing the accuracy of the classification system. These types of experiments, comparing fibres from groups of the same fibre colour/type combination, are similar to the colour block studies performed previously with textile fibres by fibre - but applying the previously established application of the MVA approach of the classification

system to further investigate the potential strengths and limitations of such a system [31,155].

However, it should be noted that these published colour block studies consider discriminating power; i.e. *"the number of discriminated pairs divided by the number of possible pairs"* [59], which is only similar to the scenario whereby groups of fibres originate from different sources. When groups of fibres truly originate from the same source, a model system should these groups are "indistinguishable", meaning that it would be inappropriate to refer to the outcomes of these MVA colour block experiments in same terms of "discriminating power", but more so with respect to accuracy. By reporting discriminating power, the true potential of the classification system may be falsely underrepresented.

4.7.6 Colour Block Studies

Colour block studies provide information on the ability of a suite of analysis (e.g. microscopy, followed by MSP and/or thin layer chromatography (TLC)) to discriminate between fibres of the same broad fibre/colour combination [38]. Colour block experiments are often considered the logical progression from population studies - wherein the most commonly occurring fibre type/colour combinations are identified before assessing how successfully a suite of analysis can discriminate the groups of fibres.

Discriminating power is stated having considered *all* of the comparative tests available to the examiner to attempt to discriminate the groups. As stated by Palmer, "*whilst colour block studies quantifying the discriminating power of the tests used in distinguishing between fibres belonging to a particular generic fibre type/*

colour, they do not in themselves, provide an estimate of how likely it is that a fibre of a particular morphology, colour, dye type and chemical composition will be found on a random surface by chance" [38] – this is considered by target fibre studies.

4.7.7 Previous Colour Block Studies

Over the years there have been a number of colour block studies carried out that show the improvements in discrimination power, reliability and functionality of analytical equipment [59,61,62,72,91,92,159] - with some examples of the outcomes of these studies listed in Table 12.

Table 12: Selection of previously published colour block studies

Author(s)	Year	Colour Block(s)	Discriminating Power with suite including (but not necessarily solely) MSP
Grieve *et al.* [91]	2001	Black Cotton Dyes	0.13 – 0.93 (dependent on dye)
Grieve *et al.* [92]	2003	Orange Cotton Green Cotton	0.930 0.998
Biermann [62]	2007	Blue Cotton Red Cotton	0.9996 0.9995
Buzzini and Massonnet [61]	2015	Blue Acrylic Black Acrylic Red Acrylic Blue Cotton Black Cotton Red Cotton Blue Wool Black Wool Red Wool	0.98 0.80 0.92 0.93 0.86 0.58 0.98 0.91 0.98
Palmer, Hutchinson and Fryer [59]	2009	Blue Cotton	0.89 (Vis) – 0.96 (UV-vis)
Jones and Massonnet [72]	2009	Light Blue Cotton Dark Blue Cotton	0.59 0.93
Jones and Coyle [159]	2010	Blue Nylon Flock	0.974

4.8 Previous studies utilising MVA for textile fibres - Reichard, Bartick, Morgan and Goodpaster

Reichard *et al.* [67] used MSP and a combination of hierarchical cluster analysis,, PCA and discriminant analysis to group 10 yellow polyester fibres into three broad groups based on their dye loading (i.e. low, medium and high dye loading). However, the authors observed poor accuracy (~51%) when trying to classify the 10 fibre sources into 10 different groups i.e. one group for each exemplar – similar to the block of colour scenarios presented in this chapter.

Reichard *et al.* relied on the interpretation of cluster analysis (both from hierarchical cluster analysis and visual examination of PCA data and clusters) and therefore does not provide an objective methodology with minimal user input as desired for an ideal classification system. Furthermore, the poor ability to discriminate fibres based on their MSP data outside of identifying the three broad groups of low, medium and high dye loading fails to accurately, and probabilistically, address one of the main questions of our research - are two fibre groups indistinguishable or distinguishable.

4.9 The need for change and advancement

As technology and equipment has progressed, increased discrimination, while measuring smaller samples, has become possible. However, even the most sensitive discriminating analytical technique is rendered to be little value if its results cannot be applied to answer specific case related questions [33,160–162].

The studies of Grieve *et al.* [108] and Grieve, Biermann and Davignon [91] assessed the discrimination of the most commonly encountered coloured cotton

fibres (black blue, red). These studies illustrated that microscopy alone offered very poor discrimination, but that this was considerably increased when visible range MSP was carried out. In the years following this study, more discriminating instrumentation capable of operating from the visible into the UV range was introduced into many operational forensic laboratories [38]. The results obtained by Biermann [62] showed that the discrimination afforded by UV-vis range MSP in combination with microscopy provided meant groups of red and blue cotton fibre types could be reliably distinguished – something which had been very difficult previously.

This is something worth considering should the current approach prove to provide insufficient accuracy, particularly when trying to discriminate fibres from the same colour block. The additional information provided by extending MSP to the UV range may aid the classification system in discriminating these samples by giving a larger dataset to interpret which may contain information pertaining to differences between samples which truly originate from different sources [68,69]. However, in the first instance for the resrach presented in this thesis, UV range MSP was not considered as this represents higher equipment and consumable costs to a forensic provider, meaning that from a cost aspect if a technique can be developed that only requires visible range MSP then this would be advantageous.

Further investigation into the discrimination of blue cotton fibres by UV-vis MSP alone, by Hutchinson *et al.* [59], not only corroborated the results of the previous studies regarding the discrimination of blue cotton, but also provided scientific justification for modifying the scheme of analysis - to use MSP as the ‘first test’ for blue cotton rather than the previous dogma of beginning with visual examination and microscopy *before* considering MSP and more destructive techniques such as

TLC. These conclusions were also supported by Buzzini and Massonnet [163] who investigated the discrimination power of particular analytical methods when employed in comparisons using a number of different coloured acrylic, wool and cotton fibres. Their study illustrated that the optimal analytical sequence differed according to which particular fibre type and colour combination is under consideration.

In the view of Palmer [38] "*many of the approaches to fibre examination still carry over thinking and dogma which predates these technological developments".* Given the drive to decrease turnaround times and provide more robust interpretation in fibre examinations, as exemplified by Grieve and Wiggins [104], and given that the discriminating power of microscopy alone in the comparison of the most commonly encountered cotton fibres has been shown to be of limited value [22], rethinking and questioning the current dogma, that the application of microscopy should always be used as the 'first test' in a fibre comparison sequence, is greater than ever.

In this research, although consideration is given by the user as to the visual comparison of two groups of fibres that are being investigated, the MVA techniques being investigated (i.e. PCA-LDA and LDA-own) are unable to consider these visual observations and rely solely on the provided MSP spectra. Therefore, the proposed MVA approach in this research would complement the argument for considering a new examination pathway, utilising MSP as a first test, particularly when examining blue, red and black cotton fibres.

From Table 3 the discriminating power observed when examining blue, black/grey and red cotton fibres using traditional methods are 0.59 to 0.99, 0.13 to 0.93 and 0.58 to 0.99 respectively.

4.10 Conclusions

A thorough set of experiments was performed to determine the optimal settings for the model classification system that allowed for high classification accuracy regardless if the two groups of fibres, based on their dyes, originated from the same or different sources. This set of experiments was successful, as regardless of which MVA approach was utilised, the highest classification accuracy was observed using the same upper/lower SPP and E.P. The number of fibres per group did appear to have an influence on the classification accuracy, as by increasing the number of fibres per group from five to ten improved the classification accuracy.

The optimal settings, based on those which provided the highest classification accuracy, were determined to be:

- **Number of fibres per groups = 10**
- **Upper/Lower self-predicative probability (SPP) = 0.9999/0.0001**
- **Exceedance Proportion (E.P.) = 0.5**

This has allowed for the early establishment of the optimal settings when considering the limitations of the proposed system with other settings which resulted in unsuitable (<90%) classification accuracy. However, this was in a very simple scenario where the groups of fibres were obviously indistinguishable or

distinguishable and therefore the classification system (and a fibre analyst) should both be successful at making correct recommendations.

These settings will therefore be used going forward to other experiments investigating blocks of colour, limits of detection, and single fibre scenarios wherein the limitations and applications of such a classification system can be examined more compressively.

5. The Application of Multivariate Analysis to Colour Block Scenarios

5.1 Aims and Objectives

The aim of this chapter is to assess the accuracy of the previously determined optimal settings for the classification system when working with groups of fibres of commonly encountered broad colour/fibre type combinations – mimicking the scenario encountered by the fibre examiner. Blue, red and black/grey cotton fibres were examined as these represented three of the most commonly encountered type/colour combinations in the published population studies and in casework and means that comparisons being made are between more visually and spectrally indistinguishable samples.

To meet this aim, the following objectives will be considered:

1. Investigate the accuracy of the classification using the settings determined as optimal in the previous chapter when comparing colour block from **different sources**.
2. Investigate the accuracy of the model classification using the settings determined as optimal in the previous chapter when comparing colour block from the **same sources**.
3. Evaluate the limitations of the proposed classification system, and investigate potential resolutions to any limitations encountered.

5.2 Methodology

5.2.1 Fibre Selection

The model classification system was evaluated using red, blue and black/grey cotton fibres as these represented the most common colour/fibre type encountered in fibre population studies and in forensic casework [5,7–9,11,21,156–158].

In these selections of groups of fibres, samples were taken from both groups that originated from the same and different sources, with the true origin of the fibres being unknown to the MSP user until after the decisions had been made by the system. This third party involvement was to ensure there was no bias from the MSP user when selecting the fibres, collecting the data and during the subsequent interpretation of the classification system output. The accuracy of the classification system was then evaluated by considering the number of “excluded” recommendations when the two groups of fibres originated from different sources, as well as the number of “indistinguishable” recommendations when the two groups of fibres originated from the same source.

5.2.2 Mounting fibres and obtaining MSP data

Cotton fibres were scraped from the surface of a fibre shade card and mounted in phytohistol on glass slides. The slides were labelled in such a way that the true origin of the fibres was unknown to the MSP user (e.g. Red 1, Red 2, Red 3 etc.). MSP was then performed in the VIS range (380 – 710 nm), taking readings from 10 fibres from each source. Each of these 10 fibres had three reading taken along their length before the three readings were averaged to produce an average spectrum for each fibre [41,136], as before. Three readings were taken along the length of each fibre before being averaged to try and minimise the effect of intra

sample variation caused by variation in dye uptake along the length of the fibre – something that is more common in natural fibres such as cotton [3,29,58,85].

5.2.3 Settings used for the classification system

- Upper/lower SPP = 0.9999/0.0001
- Exceedance proportion = 0.5
- Number of fibres per group = 10

5.3 Results & Discussion

In this chapter two situations were considered. The first situation used fibres of the same block of colour (i.e. same broad colour and fibre type) but originating from different sources, and the second used fibres from the same source. These situations reflect the question asked of fibre examiners and allow us to assess the accuracy of the proposed MVA techniques at addressing the question given the expected recommendation - i.e. can the proposed MVA technique correctly recommend two groups of fibres as being “excluded” when they originate from different sources and “indistinguishable” when they originate from the same source.

5.3.1 LDA-own – Fibres from different sources

LDA-own was utilised with the datasets first, as this MVA approach demonstrated the highest overall accuracy when using the optimal settings during the experiments reported in the previous chapter. Black/grey, red and blue cottons were investigated as these represented three of the most commonly encountered fibre colour/type combinations in the published population studies. Each of these experiments resulted in 100% classification accuracy.

This was anticipated as LDA-own, which utilises LDA for both dimension reduction and subsequent classification, looks to maximise discrimination in the dataset – therefore in theory allowing for high accuracy when working with groups of fibres that originate from different sources. This 100% recommendation accuracy means that the model classification system, when utilising the optimal settings determined in the previous chapter, is still suitable when comparing groups of fibres that are more visually and spectrally similar than those used previously - increasing the

difficulty of analysis and therefore more closely representing the scenario and question presented to the fibre examiner.

5.3.2 LDA-own – Fibres from same sources

LDA-own was next evaluated with groups of fibres from the same source but mounted on different slides: akin to a situation where two fibre samples have been recovered from a known and questioned source of the same origin. Black/grey, red and blue cottons were again investigated for the reasons stated previously, with the results from these colours blocks demonstrated in Figure 29.

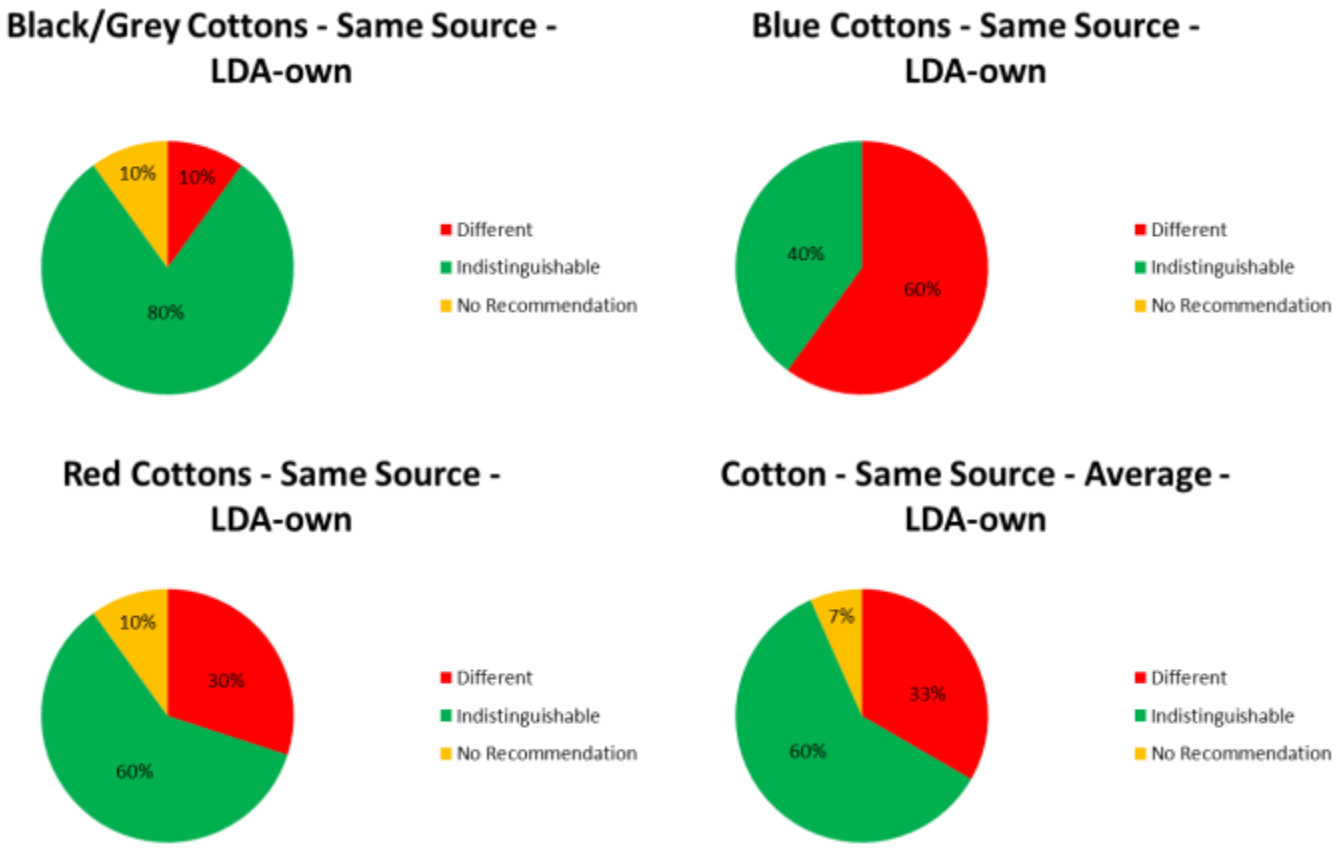

Figure 29: The percentage of each recommendation for red, blue and black/grey cotton colour blocks when using LDA-own alongside the optimal settings for fibres from same sources

When considering groups of fibres from the same source, LDA-own was unable to demonstrate suitable (>90%) accuracy when considering any of the blocks of colour. Black/grey cotton showed an 80% correct recommendation accuracy, blue cotton showed 40% recommendation accuracy and red cotton showed 60% recommendation accuracy, resulting in an overall average of 60% classification accuracy. This outcome was substantially lower than the 96% recommendation accuracy observed in the previous chapter when considering cotton samples from the same source.

This could be a result of the inherent difficulties in working with natural fibres, such as cotton may exhibit changes in dye uptake and concentration along their length due to their structure [29,58]. These small changes in dye uptake could then result in differences in the spectra that are being detected and incorrectly interpreted by the LDA-own method due to the sensitivity of the technique. When being sampled and mounted separately, these changes in dye uptake may then result in a non-representative sample being used, at least from the point of the view of the LDA-own technique.

Example spectra from red (Figure 30), blue (Figure 31) and black/grey (Figure 32) groups of fibres which were erroneously decided to be “excluded” are shown, with the fibres from the first group of 10 being shown with red lines, and the second group of 10 fibres being shown with black lines.

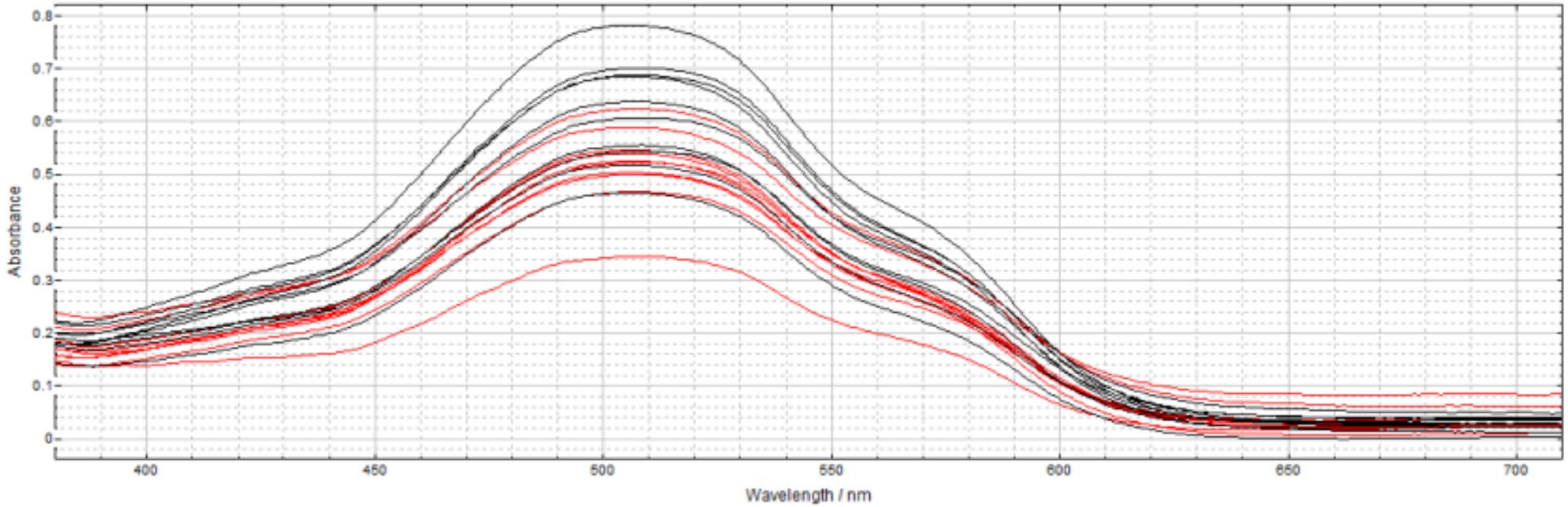

Figure 30: Example of two groups of red fibres which were erroneously recommended as being "excluded". Fibres 1-10 in red, Fibres 11-20 in black

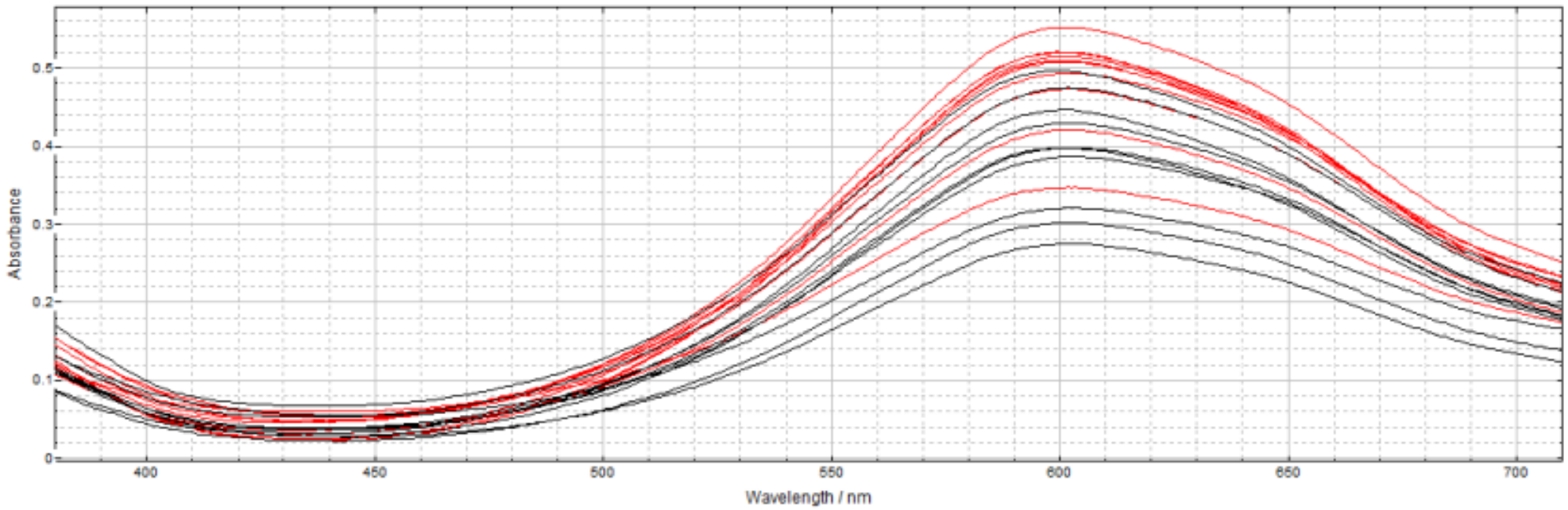

Figure 31: Example of two groups of blue fibres which were erroneously recommended as being "excluded". Fibres 1-10 in red, Fibres 11-20 in black

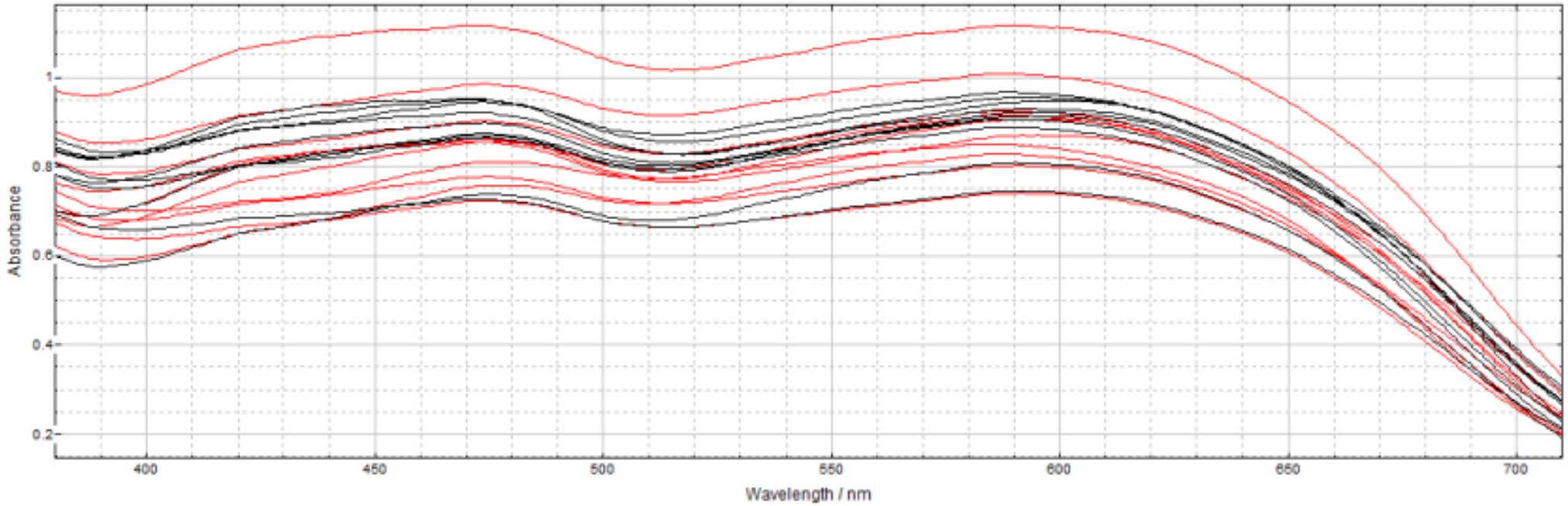

Figure 32: Example of two groups of black/grey fibres which were erroneously recommended as being "excluded". Fibres 1-10 in red, Fibres 11-20 in black

5.3.3 LDA-own - summary

LDA-own showed 100% recommendation accuracy when working with groups of fibres that originated from different source. However, when working with groups of fibres that originated from the same source, this accuracy dropped to 60% on average across the three colour blocks.

5.3.4 PCA-LDA – Fibres from different sources

Given the limited success of LDA-own when considering the same source scenario, it was decided to examine the accuracy of PCA-LDA and determine if this technique may be more suitable to both situations in these set of experiments. The above experiments were therefore repeated, with the results from comparing groups of fibres from different sources when utilising PCA-LDA with the colour blocks shown in Figure 33.

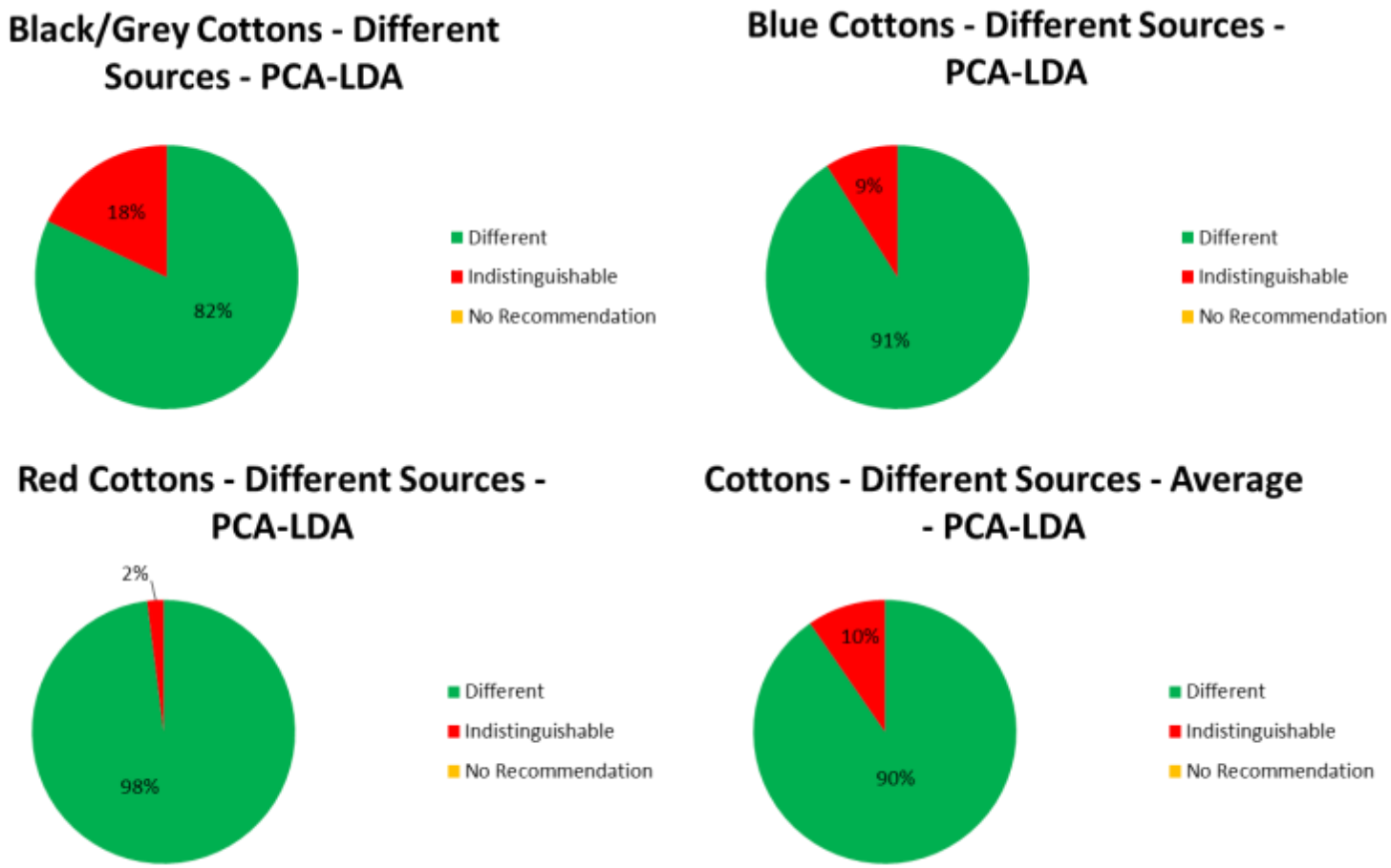

Figure 33: The percentage of each recommendation for red, blue and black/grey cotton colour blocks when using PCA-LDA alongside the optimal settings for fibres from different sources

The results in Figure 33 demonstrate that PCA-LDA, when used with groups of cotton fibres from different sources, showed a correct “excluded” recommendation accuracy of 82%, 91% and 98% when using black/grey, blue and red cotton fibres respectively – resulting in an average correct recommendation in 90% of outputs.

Black/Grey cotton fibres showed the lowest accuracy at 82% and may be due to the spectra being obtained tending to be quite broad and featureless (Figure 34). Similar accuracy has been observed in some previous colour block studies utilising black cotton – depending on the dye type and the suite of analysis. This lack of features could cause issues with the PCA-LDA technique, as there may be less useful underlying information within the MSP spectra to perform meaningful dimension reduction – increasing the potential for more noise to be included in the reduced dataset; minimising the discrimination capabilities of the system

Future examinations should consider the application of the model classification system to black/grey cotton fibres as a potential limitation and may not be reliable. However, the classification system seems to be much more reliable when considering red and blue cotton fibres.

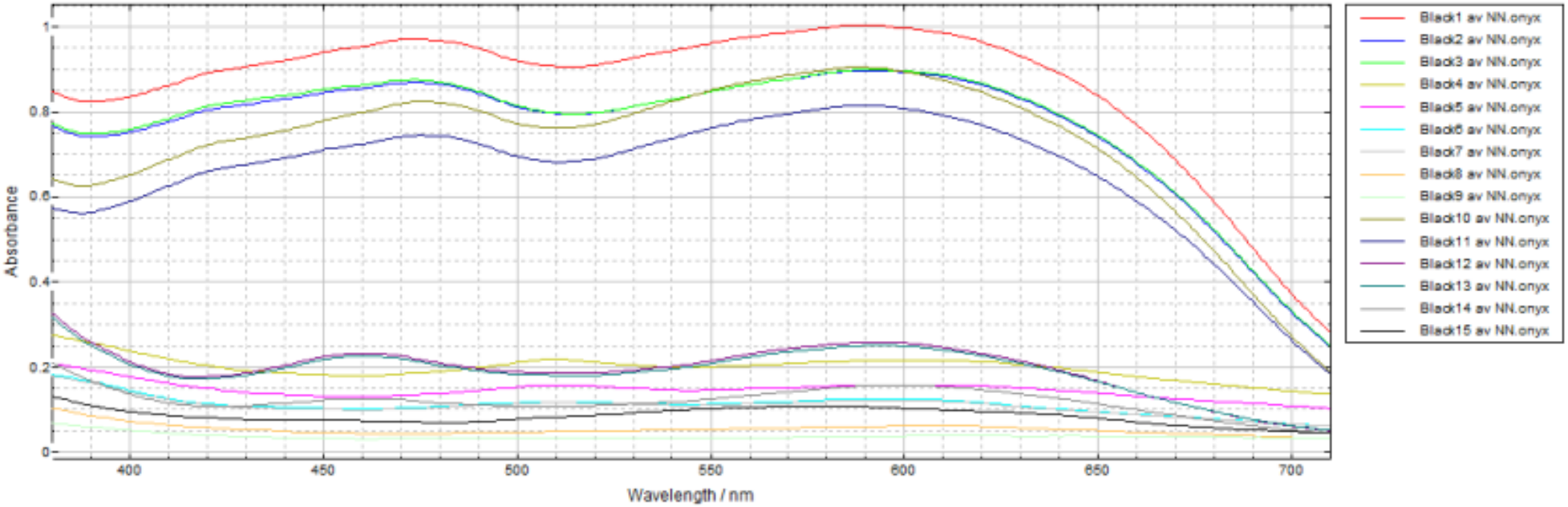

Figure 34: The average spectra produced from each of the different black/grey cotton fibres

5.3.5 PCA-LDA – Fibres from same sources

Following on from the comparisons of groups of fibres from different sources, PCA-LDA was then evaluated when examining groups of fibres from the same source. This is where LDA-own previously showed limited recommendation accuracy, with a mean accuracy of 60%. PCA-LDA showed an average accuracy of 90% when considering groups of fibres from different sources. Each of these experiments now yielded 100% classification accuracy.

These results demonstrate that PCA-LDA showed 100% recommendation accuracy for all three colour block, and therefore also on average. Because PCA looks for variation within the dataset during the dimension reduction stage, it was envisaged that it would prove to be more successful than LDA-own when trying to group together groups of fibres from the same source – results that were corroborated by the results from the previous chapter also.

Therefore, overall, PCA-LDA outperformed LDA-own when considering its application to both scenarios (different and same sources) simultaneously and would be determined to be the optimal technique when working with these more challenging and more casework-like samples. However, these results can only responsibly be considered for cotton fibres - as when considering the results from the previous chapter wherein acrylic fibres were utilised, PCA-LDA showed 99.9% accuracy when working with groups of fibres from the same source, but only 79.9% accuracy when working with fibres from different sources. It may be that therefore the fibre type needs to be established before deciding which MVA approach to utilise – with PCA-LDA showing better overall accuracy and robustness with cotton fibres, and LDA-own being more suited for acrylic fibres.

Reichard *et al.* [67] used MSP and a combination of hierarchical clustering, PCA and discriminant analysis to attempt classify the 10 fibre sources into 10 different groups i.e. one group for each yellow polyester fibres, however, the authors observed poor accuracy (~51%). The poor ability to discriminate fibres based on their MSP data fails to accurately and probabilistically, address one of the main questions of this research: are two fibre groups indistinguishable or distinguishable? In this research, up to 100% classification accuracy when trying to discriminate groups of red, blue and black/grey cotton fibres within their own broad colour group. These results suggest the proposed classification system provides a more accurate approach to differentiating fibre dyes.

5.5 Conclusions

The aim of this chapter was to move towards using more visually and spectrally similar textile fibres by utilising colour blocks, in order to create more challenging scenarios that would be more likely to cause dispute between fibre examiners and test the effectiveness of the proposed model classification system. For the blocks of colour experiments, blue, red and black/grey cotton fibres were used as these represented three of the most commonly encountered fibre colour/type combinations encountered in the available population studies.

When comparing groups of fibres from the different sources, LDA-own had 100% classification accuracy. However, when considering groups of fibres from the same source the accuracy dropped to 60%. Therefore, LDA-own was unable to successfully address both scenarios and PCA-LDA was investigated.

When comparing groups of fibres from the different sources, PCA-LDA had 90% classification accuracy – with black/grey fibres being most problematic with 82% accuracy. When considering groups of fibres from the same source the accuracy increased to 100%. Therefore, PCA-LDA was overall successful in addressing both scenarios – but the potential limitation of examining black/grey cotton fibres must be considered and it cannot currently be recommended to apply PCA-LDA to these examinations.

The fibre type (e.g. acrylic or cotton) also appears to have some influence of which MVA approach, PCA-LDA or LDA-own may be most appropriate. Whenever considering the results of both this chapter and the previous chapter, PCA-LDA is most appropriate and robust when interpreting cotton fibres, and LDA-own is more appropriate and robust when interpreting acrylic fibres (see previous chapter) –

however this latter statement would require further testing and examination to truly validate this claim to the same extent; utilising samples that are more visually and spectrally similar as was the case for the cotton fibres in this chapter and utilising other synthetic and natural fibre types.

6. Limits of Discrimination and Single Fibre Scenarios

6.1 Introduction

The previous chapters have established the optimal settings for the proposed classification system – utilising acrylic and cotton fibres that originated both from the same and (visually and spectrally) different sources. These optimal settings were then tested further utilising blocks of colour, wherein fibres of the same colour/fibre type combination are compared, thus increasing the difficulty of the analysis and moving more towards a more realistic, casework-like scenario.

The results of these previous chapters determined that the optimal upper/lower self-predictive probability (SPP) thresholds were 0.9999/0.0001, the optimal exceedance proportion (E.P.) was 0.5, and the optimal number of fibres to use for each group was 10. Principal component analysis (PCA) followed by linear discriminant analysis (LDA), hereby referred to as PCA-LDA, provided higher recommendation accuracy when working with cotton fibres, whereas LDA-own (i.e., using LDA for dimension reduction *and* classification) provided higher recommendation accuracy when working with acrylic fibres. The fibre type, if not know beforehand, can readily be established through the use of microscopy, including polarised light microscopy [4]. The fibre type does not affect the obtaining of spectra through microspectrophotometry (MSP) and therefore recommendation accuracy is more dependent on the settings for the classification system; including the multivariate analysis (MVA) approach used.

This chapter aims to build further upon the results from the previous chapters by considering two further problems encountered by the forensic fibre examiner: a) how sensitive is the proposed classification system (i.e. what percentage of dye change is required in order to provide a correct “excluded” recommendation) and b) how the model classification system performs when only single fibres are

available to be used as the questioned source. The purpose of this set of experiments was to determine the limitations of the methods.

Previous studies looking at the discrimination of textile fibre have involved the use of a suite of analysis (i.e. multiple stages and types of analysis) in order to maximise discrimination e.g. following the traditional examination pathway of visual examination followed by comparison microscopy, MSP, and then additional techniques such as spectra alteration (e.g. 1st derivative) [58,85,164] or destructive techniques (e.g. thin layer chromatography (TLC) [29,61,69,165,166]). To increase the discrimination, ultra violet (UV) range MSP can be included using MSP [29,58,59,61,62,68,69,91,110,154,167] whereby information is gathered at wavelengths below 380 nm, as has been used in this study to date. This however increases equipment costs for a forensic laboratory due to the more specialised equipment being required to have light sources suitable for the UV range but also the additional provision of quartz microscope slides and cover slips as glass readily absorbs in the UV range electromagnetic radiation [4]. Therefore, visible range was considered first and foremost as it was deemed to be the more accessible and cost effect technique available to forensic providers – thus increasing its potential application to casework.

This inclusion of the UV range, to provide UV-vis range (280 nm – 710 nm) MSP is investigated to determine its effect on recommendation accuracy with the proposed classification system.

6.1.1 Previous studies utilising MVA for textile fibres

6.1.1.1 Sauzier, Reichard, Bronswijk, Lewis and Goodpaster

Sauzier *et al.* [71] published work with the aim of addressing the questioned versus known comparison of fibre evidence using MSP data for “visually similar” blue acrylic fibres. “Similarity or dissimilarity” of each pair was determined by Sauzier *et al.* using PCA, discriminant analysis and Fisher’s exact test for independence. In their research, if there is no correlation between two groups of data then they are indistinguishable [168]. Fisher’s exact test is suitable when small sample sizes are encountered, however it does not use a mathematical function that estimates the probability of a value of a test statistic the same way that SPP does which utilises a more probabilistic approach.

Sauzier et al. took five MSP readings across 10 fibres from each source; resulting in 50 spectra in total for each blue acrylic source. The first 45 spectra (i.e. MSP data from the first nine fibres from each blue acrylic source) were used as the “known” group and the final five spectra (i.e. the five spectra from the tenth blue acrylic source) as the “questioned” group. PCA followed by discriminant analysis was used to recommend if these comparisons were “inclusions or exclusions” (i.e. indistinguishable or distinguishable). This approach of taking multiple readings from one fibre to constitute a dataset is something considered in this chapters when considering the application of the classification system to single fibre scenarios.

Sauzier et al. used MSP in the 400 – 800 nm range; similar to the 380-710 nm range used in this research. Also, a similar experimental design was also demonstrated; first taking a scenario where groups originated from the same

source, followed by an exhaustive pairwise comparison using all available samples. To make their recommendation, Sauzier *et al.* required at least three of five (i.e. a 0.6 exceedance proportion as it would be called in this research) of the spectra from the tenth fibre to be inclusive to the first 45. Sauzier *et al.* reported 10 of 11 correct “inclusion” results and 108 of 110 “exclusion” results; however the obvious argument is sample size – particularly for the inclusion scenario. In addition, the limited sources examined (only blue acrylic was considered) means that much more work would be required from this approach to determine the feasibility and robustness of such an approach to the wider and more common (when considering fibre population studies) fibre population.

6.1.1.2 Sharma, Kumar and Kaur

Sharma *et al.* [154] analysed dyes extracted from cotton and wool fibres through a variety of solvent systems using UV-vis spectrophotometry (200 – 800 nm). Visual comparison of the peaks obtained by spectrophotometry allowed ~84% of cotton fibres and ~94% of woollen fibres to be successfully discriminated. This number was report to have increased to 100% for cotton fibres and ~98% for woollen fibres following the application of PCA and Welch’s t-test.

However this study only examined eleven cotton fibres and fifteen woollen fibres – an obvious sample size issue. Additionally, the use of dye extraction from fibres prior to analysis constitutes a destructive approach – meaning that the fibres will no longer be able to be examined by other means. The use of MSP in this research in a much more non-destructive approach – meaning that the evidence is not destroyed and can still be used for further analysis or stored for future reference [78]. Furthermore, only the discrimination of fibres is considered, and not

the ability of the proposed method to determine if two groups of fibres that truly could have originated from the same source are not falsely excluded.

6.2 Aims and Objectives

The aim of this chapter is to assess the capabilities of the proposed model classification system when considering some of the most challenging scenarios encountered by a fibre analyst; small dye changes between mixtures utilising own-dyed cotton samples and when only a single fibre from a questioned source is available for analysis.

The objectives of this chapter are:

- Assess the classification accuracy of both PCA-LDA and LDA-own when working with own-dyed cotton fibres that originate both from same and different dye mixtures; where the dye percentage can undergo small and measured changes between mixtures.
- Determine the limit of discrimination of the proposed model classification system i.e. what range of percentage difference between dye mixtures must be present in order for the system to successfully return an “excluded” recommendation.
- Evaluate if the use of UV-vis range MSP increases the recommendation accuracy for the above scenarios compared to Vis range MSP alone.
- Assess the suitability of the model classification system in terms of recommendation accuracy when presented with a single questioned fibre to be compared against a group of known fibres.

6.3 Methodology

6.3.1 Obtaining and Preparing Cotton for Dyeing in house (own-dyed)

Pre bleached, white, 100% cotton fabric was obtained from an online haberdashery (John Lewis). Before dyeing, the fabric was washed in a domestic washing machine at 60 °C using Daz™ biological washing powder (Procter & Gamble) without any fabric softener to remove any finishers that were still present on the fabric obtained from the manufacturer before being air dried.

Once dried the fabric was cut into squares (each approximately 5 cm x 5 cm and weighing approximately 0.42 g).

6.3.2 Dyeing Cotton

To ensure the correct ratio of fabric to liquid (i.e. liquor ratio), 24 squares of fabric were dyed in each mixture - given a total fabric weight of approximately 10 g. This weight was selected so that a liquor ratio i.e. the ratio of fabric weight (g) to volume of liquid (mL), of 1:20 could be used conveniently – meaning for dyeing each 10 g of fabric, 200 mL of liquid was used. This 1:20 liquor ratio which is within a range of commonly encountered liquor ratios for dyeing cotton [169,170].

A methodology for direct dyeing of cotton fabric was obtained from a person with previous experience in a dye house [171]. The cotton was dyed using mixtures of Direct Red 23 (DR23) (BDH) and Direct Blue 6 (DB6) (BDH). Direct dye was selected due to its straightforward dyeing process suitable for performing in the lab without the need for highly specialised equipment as would be required for some other dyeing processes. Red and blue dyes were selected due to their abundance in the general fibre population [6–9,11,15,21–23]. The overall concentration of dye

used in each dye mixture remained the same (0.1% w/v) with the only change in each being the ratios of DR23 and DB6. A full list of the dye percentages used in each sample is listed in Table 13.

Table 13: A full list of the dye percentages used for each sample in the own-dyed experiments

Dye Mixture Variate	Percentage DR23 (%)	Percentage DB6 (%)
1	100	0
2	95	5
3	90	10
4	85	15
5	80	20
6	75	25
7	70	30
8	65	35
9	60	40
10	55	45
11	50	50
12	45	55
13	40	60
14	35	65
15	30	70
16	25	75
17	20	80
18	15	85
19	10	90
20	5	95
21	0	100

To dye the cotton samples, 200 mL of tap water was brought to the boil over a Bunsen burner (Better Equipped Educational Supplies Ltd) in a 500 mL beaker. The required amounts of DR23 and DB6 for each dye percentage was then added and solubilised using a glass stirring rod. The dye solution was cooled to approximately 50°C before the undyed 5 cm x 5cm fabric squares were dampened with tap water and added to the beaker containing the dye solution. The solution was then brought back to the boil before adding 2 g of sodium chloride (Sigma Aldrich). The solution was then held at boiling temperature for approximately 30 minutes.

The temperature was reduced to approximately 80°C by adding cold tap water to the beaker containing the dye solution and held at approximately 80°C for approximately 15 minutes. Finally, the dyed fabric was removed from the beaker and gently rinsed under running tap water until the water ran clear (i.e. removing any excess, unbonded dye) before samples were air dried on a drying line in a dark room. A dark room was used as during the initial experiment poor light fastness of the dyed fabric was observed and photo bleaching was visually observed on samples air dried beside a large window overnight (a phenomenon presented by Forster *et al.* [28]) and so the dyeing process was restarted and a dark room for drying included..

The resulting, dried, dyed cotton samples are shown in Figure 35, with the numbers underneath referring to the percentage of DR23 and DB6 used in each dye mixture. From left to right, top to bottom, the dye proportions of each dye for the 0.1% w/v changes from 100% DR23 to 100% DB6, with a 5% change in both dyes between each dye mixture. For example, the top left sample contains 100% DR23 and 0% DB6. The sample to the right of this contains 95% DR23 and 5%

DB6. The sample to the right of that contains 90% DR23 and 10% DB6 and so on until 0% DR23 and 100% DB6 was used.

Figure 35: The 21 different cotton samples created during the own dyed cotton experiments

A sample of three fibres from each swatch in one dye mixture (100% DR23) was analysed using the visible range MSP protocol to ensure that even dyeing had occurred. The results of this analysis (Figure 36) shows that similar shape spectra were produced from each swatch; suggesting that even dyeing had occurred and there were no significant inter-sample variation observed.

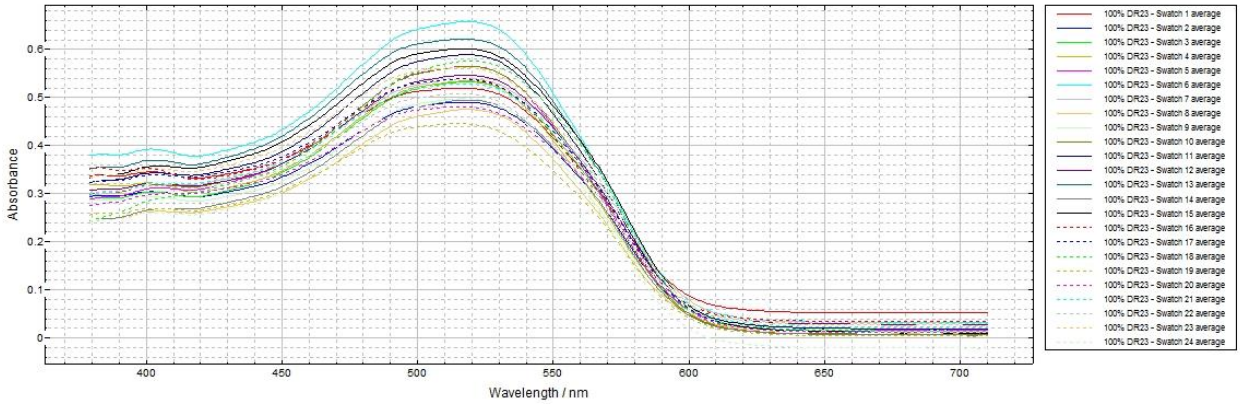

Figure 36: Average spectra produced by fibres from each of the 24 swatches in the 100% DR23 dye mixture

Of these 24 dyed cotton samples for each dye mixture, five (the approximate square root of 24 [172]) were selected for subsequent MSP analysis. Sample 1 was selected from near the top of the dye bath, sample 5 was selected from near the bottom of the dye bath, and the remaining samples were selected at random intervals between these to ensure that the depth of the sample in the dye bath did not appear to have an effect on the subsequent results.

Fibres were then scraped from the surface and mounted onto the appropriate slide type (i.e. glass for Vis range MSP, and quartz for UV-vis range MSP) before MSP examination; using the protocol for Vis range or UV-vis range as listed in chapter two. Any slides with mounted fibres, as well as the dyed cotton samples used were kept in a dark cupboard and in a separate brown paper bag for each dye mixture

composition when not being examined to reduce any unnecessary photo bleaching or cross contamination from occurring [28].

6.3.3 Microspectrophotometry of single fibres

For the single fibre scenarios, a single fibre was randomly selected from each slide to be analysed using MSP. The only selection criterion was that the fibre had to be long enough to have 10 readings taken along its length. 10 readings were taken along the length of the single fibre to simulate the “fibres per group = 10” optimal setting previously established for the model classification system. This approach simulates a scenario whereby only a single fibre is available for analysis, and not the minimum ten as recommended by the European Textile and Hair Group Guidelines [41]. However, such an approach has been utilised before in the Stephen Lawrence case when examining a single red fibre [173].

6.3.4 Settings for Model Classification System

The following settings were used, based upon the optimal recommendation accuracies observed in the previous chapters of this research:

- Upper/Lower SPP = 0.9999/0.0001
- Exceedance Proportion = 0.5
- Number of fibres per group = 10

6.4 Results and Discussion

6.4.1 Own-dyed cotton

Fibres from the five samples taken from each different dye mixture were compared in a pairwise manner until all unique combinations had been exhausted to determine if these could be successfully recommended as being "indistinguishable". Using fibres from five samples from each dye mixture resulted in ten comparisons for each of the different dye mixtures (i.e. samples 1 vs. 2, 1 vs. 3, 1 vs. 4, 1 vs. 5, 2 vs. 3, 2 vs. 4, 2 vs. 5, 3 vs. 4, 3 vs. 5 and 4 vs. 5). Therefore, with 21 different dye percentage compositions (e.g. 10% DR23, 90% DB6) and ten comparisons per dye mixture the total number of recommendations made using each MVA approach was 210 (i.e. 21 x 10).

6.4.2 Own-dyed cotton – Vis range MSP– PCA-LDA

Principal component analysis for dimension reduction of the dataset, followed by linear discriminant analysis for classification (PCA-LDA) was utilised first as in the previous chapters this proved to be the optimal MVA approach when working with cotton fibres and Vis range MSP; specifically when considering overall recommendation accuracy for both scenarios where fibres have originated from the same and from different sources.

6.4.2.1 Same Dye Mixture – Vis Range MSP - PCA-LDA

When using PCA-LDA, eight of the 210 recommendations were incorrect (i.e. "excluded" or "no recommendation") - resulting in a recommendation accuracy of 96% (Figure 37).

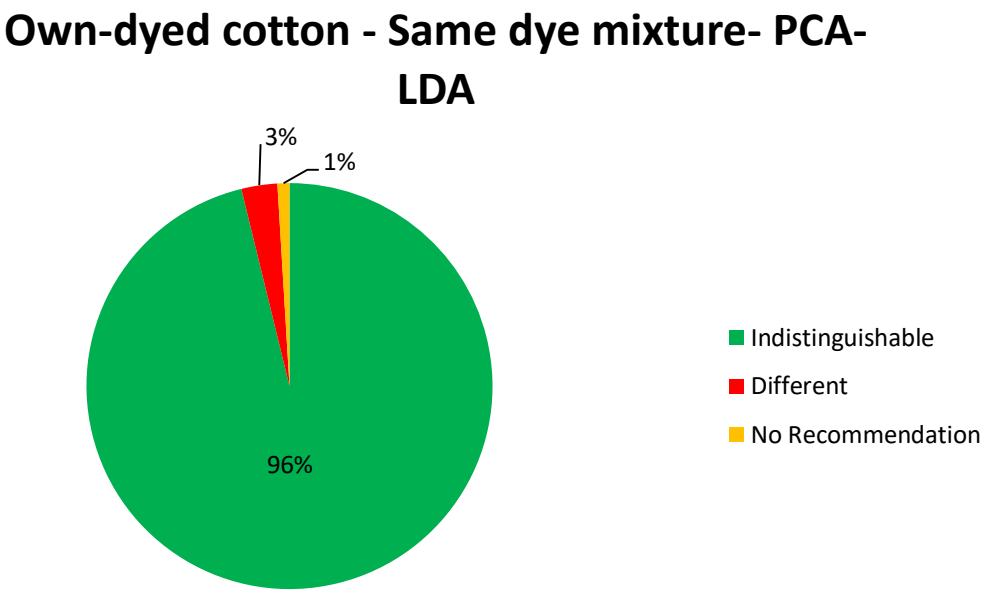

Figure 37: The average recommendation percentages when using own dyed cotton, SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibre= 10 when comparing groups of fibres from the same dye mixture using PCA-LDA

This suggests that the previously determined optimal settings for the model classification system continues to be able to successfully group together fibres from the same source when using own-dyed cotton as similar average recommendation accuracies had been observed previously (100% accuracy in chapter four, and 100% accuracy in chapter five) and that depth of each sample in the dye mixture did not seem to negative effect the recommendation accuracy – implying a relatively uniform dyeing process in each dye mixture.

Following this, groups of fibres from different dye mixtures were compared to determine the limits of discrimination of the PCA-LDA approach.

6.4.2.2 Different Dye Mixtures – Vis Range MSP - PCA-LDA

Utilising a pairwise approach to compare each of the 21 different dye mixtures until all possible unique combinations were exhausted resulted in 210 comparisons. Because five samples from each dye mixture had been sampled and examined using MSP, this resulted in a total 1050 recommendations (210 recommendations per sample x 5 sample per dye mixture) being made. Figure 38 shows the overall recommendation percentages using the optimal settings and PCA-LDA obtained when comparing samples from different dye mixtures.

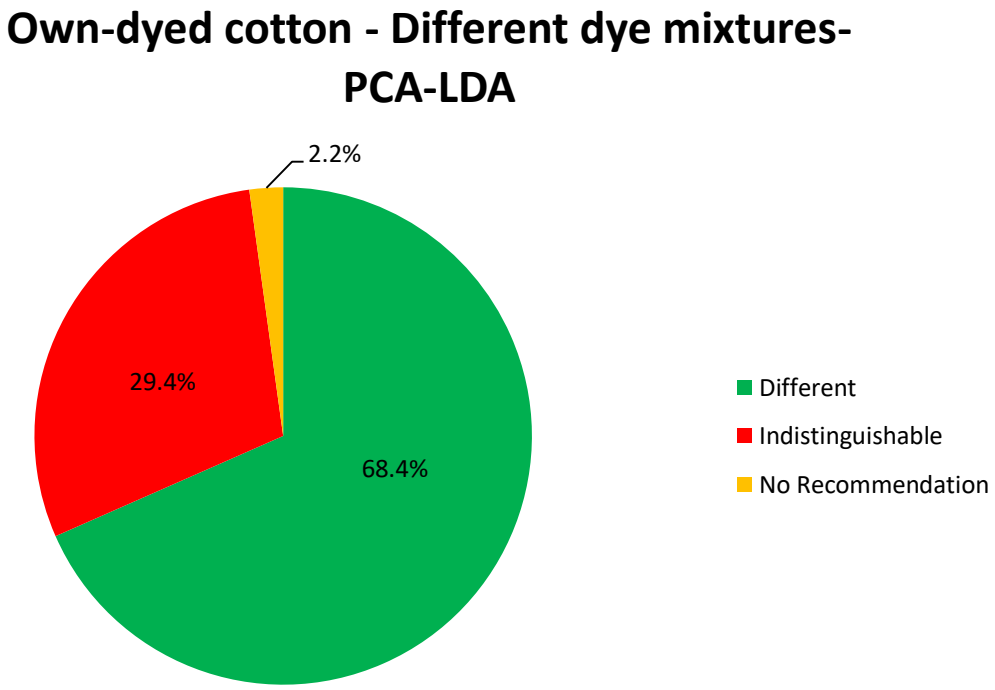

Figure 38: The average recommendation percentages when using own dyed cotton, SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibre= 10 when comparing groups of fibres from different dye mixtures with PCA-LDA

The above results show that PCA-LDA, alongside the optimal settings for the model classification system, provided a correct “excluded” recommendation 68.4% of the time when comparing groups of fibres from different dye mixtures. This

number is below the average correct recommendation rate of 90% observed in the previous chapter, when considering groups of red, blue and black/grey cotton fibres from the different sources.

This lower recommendation accuracy compared to previous may be due to the type of dye (i.e. direct dye) - which may be different than the dyes used in the previous chapters (e.g. vat, reactive or sulphur dye). This is purely speculative as the type of dye used in the previous chapters was not determined, however a similar phenomenon has been observed in a previous study involving black cotton dyes wherein the discriminating power varied from 0.13 for sulphur dyes to 0.93 for active dyes depending on the dye being investigated [91]. Direct black dyes showed a discrimination power of 0.89 but with a limited sample size (only ~11% of the samples investigated by Grieve, Biermann and Davignon [91]. However, this reduction in recommendation accuracy may also be due to limitations in the discrimination sensitivity of the PCA-LDA approach.

With regards to sensitivity and limits of discrimination, this is explained further when looking at the error heat map (Figure 39) which uses a colour scale to show the number of incorrect recommendations (i.e. “indistinguishable” or “no recommendation”) for each pairwise comparison (i.e. different dye mixtures) to examine where most errors occur; with a maximum value of 5 meaning that all five samples resulted in incorrect recommendations.

% DR23 / % DB6	100/0	95/5	90/10	85/15	80/20	75/25	70/30	65/35	60/40	55/45	50/50	45/55	40/60	35/65	30/70	25/75	20/80	15/85	10/90	5/95	0/100
100/0																					
95/5	5																				
90/10	4	5																			
85/15	3	5	5																		
80/20	1	3	5	5																	
75/25	3	5	5	5	4																
70/30	1	2	2	2	1	5															
65/35	3	2	3	2	2	5	5														
60/40	1	1	2	0	1	2	4	5													
55/45	1	2	1	1	1	2	5	5	5												
50/50	1	3	2	1	1	2	5	5	5	5											
45/55	1	0	2	1	1	1	3	4	3	5	5										
40/60	0	0	1	0	1	0	2	2	4	5	5	5									
35/65	0	0	1	0	0	0	2	2	3	4	5	5	5								
30/70	0	0	1	0	0	1	0	2	3	2	3	2	5	5							
25/75	0	0	1	0	0	0	1	2	1	1	3	2	4	5	5						
20/80	0	0	0	0	0	0	0	1	1	0	1	0	2	3	5	5					
15/85	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	2	5				
10/90	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	5			
5/95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0/100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	

Incorrect recommendations:	0	1	2	3	4	5

Figure 39: Heat map showing the frequency and occurrence of the incorrect ("indistinguishable" or "no recommendation") recommendations when using the previously determined optimal settings when comparing own-dyed cotton samples from different dye mixtures with PCA-LDA

In total, 331 of the 1050 recommendations made by the model classification system were incorrect i.e. “excluded” or “no recommendation”. When looking at the heat map in Figure 39 and breaking down these results further, the percentage of incorrect recommendations made, based on the closeness of the dye composition for each dye mixture comparison can be investigated; with the results summarised in Figure 40.

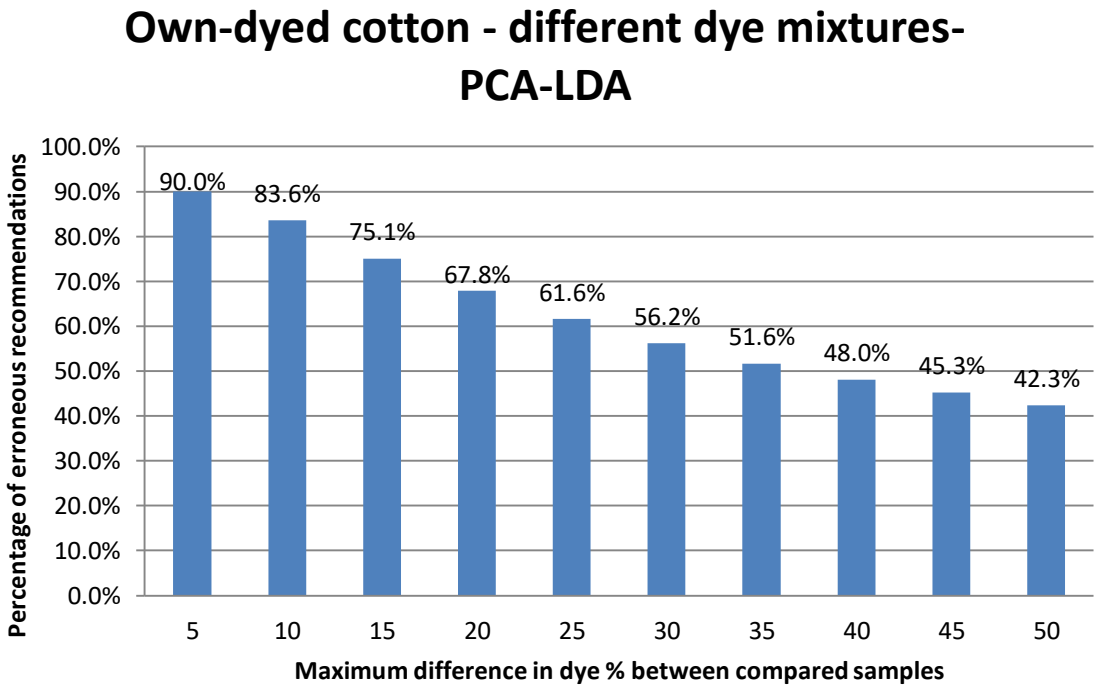

Figure 40: The percentage of incorrect recommendations when using the optimal settings with PCA-LDA when comparing own-dyed cotton samples from different dye mixtures to investigate limits of discrimination.

The above results (Figure 39 and Figure 40) demonstrate the sensitivity of the PCA-LDA method, and highlight the struggles of the technique when comparing fibres of a very similar dye composition but from different sources i.e. those with a similar dye composition. Figure 39 clearly demonstrates that the larger the difference in the dye composition between the two sources, the more accurate PCA-LDA becomes as fewer incorrect recommendations are returned. Figure 40

shows that when the difference in proportion of each dye between different dye mixtures was 5% or less that 90% of the returned recommendations were incorrect. This percentage then decreased to 83.6% when the difference between dye mixtures was 10% or less, 75.1% when the difference between dye mixtures was 15% or less and so on. This seemingly logical explanation is important to appreciate, as if the incorrect recommendations were more random, and did not seem to depend on closeness of dye composition, it could suggest that the erroneous recommendations obtained were more due to the stringent upper/lower SPP threshold and/or the proposed exceedance proportion rather than the similarity between the two groups of fibres being compared – suggesting that the previously determined optimal settings remain suitable for application.

Overall, these results demonstrate that PCA-LDA is still highly able to correctly recommend when two groups of fibres may originate from the same source. However, in terms of limits of discrimination, PCA-LDA may lack sensitivity to successfully discriminate between two groups of fibres when smaller changes in dye percentage between groups of fibres are present. In these scenarios, it may be required to perform further analysis more akin to the traditional pathway of fibre analysis; incorporating techniques such as comparison microscopy, UV-vis range MSP and destructive techniques such as TLC to maximise the opportunity to correctly and successfully discriminate the two groups of fibres.

6.4.3 Own-dyed cotton – Vis range MSP – LDA-own

The use of LDA-own (linear discriminant analysis for both dimension reduction *and* classification) as the MVA approach, as opposed to PCA-LDA, was also investigated, as the MVA approach to determine if this would be a more suitable

approach for these challenging scenarios as it had shown potential in some sections of the previous chapters.

6.4.3.1 Same Dye Mixture – Vis Range MSP - LDA-own

When using LDA-own to compare groups of fibres from the same dye mixture (Figure 41) 135 of the 210 recommendations were incorrect (i.e. “excluded” or “no recommendation”; resulting in an overall mean classification accuracy of 35.7%.

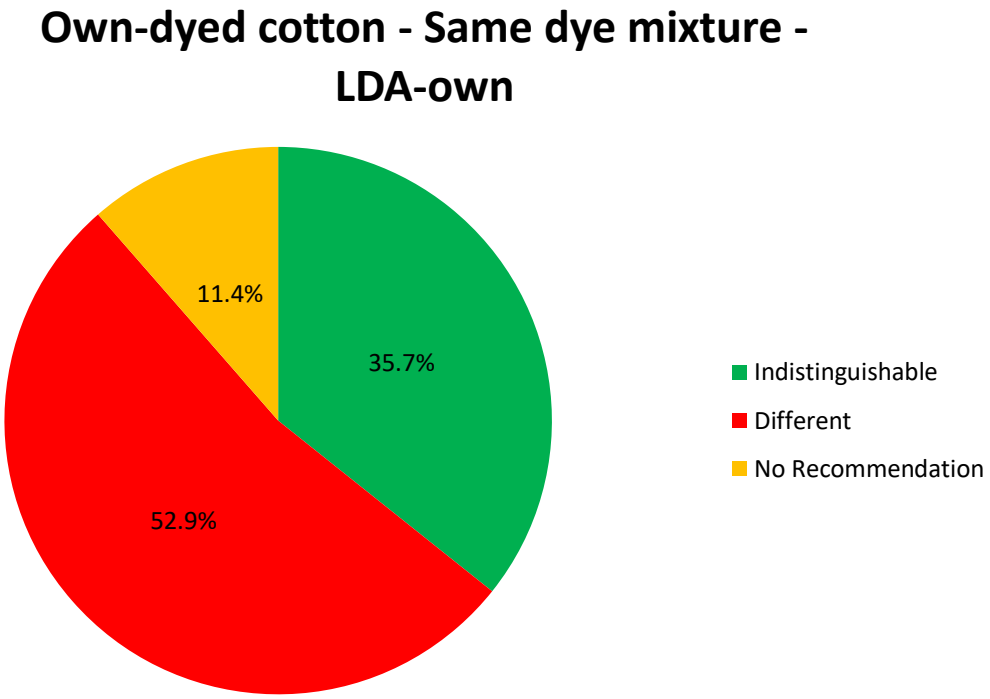

Figure 41: The average recommendation percentages when using own dyed cotton, SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibre= 10 when comparing groups of fibres from same dye mixtures with LDA-own

These results re-enforce the previous observations that PCA-LDA outperforms LDA-own when recommending that two groups of fibres are “indistinguishable” and therefore may have originated from the same source when this truly is the case. When used as a dimension reduction technique, LDA looks to optimise

discrimination between the groups in a given dataset [174]. Therefore it is likely that this dimension reduction stage is resulting in a dataset more focussed on discrimination rather than variation that subsequently does not contain the necessary underlying information to successfully recommend that two groups of fibres correctly may originate from the same source when this is truly the case.

It is also worth noting that in the previous chapter, when considering colour block experiments using blue, black/grey and red cotton, the mean classification accuracy observed using LDA-own was 33%; so it would appear that this observation is not necessarily an outlier but holds true whenever two groups of fibres of a similar colour (and therefore similar spectra) are being compared using this approach with the determined optimal settings.

6.4.3.2 Different Dye Mixtures – Vis Range MSP - LDA-own

Given the poor results observed above, when comparing groups of fibres from the same dye mixture (Figure 41), the results when considering different dye when utilising LDA-own would not address both elements of the question (i.e. match/non-match). However, when using LDA-own when comparing fibres from different dye mixtures, an average correct recommendation rate of 93.3% was observed (Figure 42).

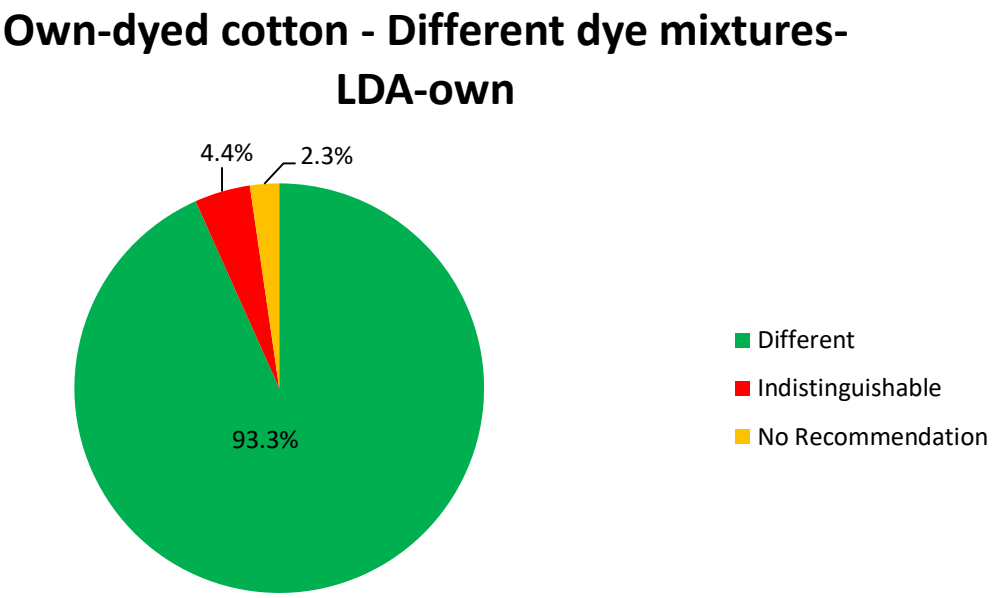

Figure 42: The average recommendation percentages when using own dyed cotton, SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibre= 10 when comparing groups of fibres from different dye mixtures with LDA-own

Overall, 6.7% (70 of the 1050) recommendations were incorrect. When examining the above results utilising a heat map (Figure 43) as previous it can be seen that 69 of the 70 erroneous recommendations occurred when the difference between the percentages of DR23 in the compared groups of fibres was 20% or less.

% DR23 / % DB6	100/0	95/5	90/10	85/15	80/20	75/25	70/30	65/35	60/40	55/45	50/50	45/55	40/60	35/65	30/70	25/75	20/80	15/85	10/90	5/95	0/100
100/0																					
95/5	0																				
90/10	0	3																			
85/15	0	0	2																		
80/20	0	1	1	2																	
75/25	0	0	1	4	4																
70/30	0	0	0	1	0	1															
65/35	0	0	0	0	0	1	3														
60/40	0	0	0	0	0	0	1	2													
55/45	0	0	0	0	0	0	2	1	3												
50/50	0	0	0	0	0	0	1	1	3	5											
45/55	0	0	0	0	0	0	0	0	2	1	3										
40/60	0	0	0	0	0	0	0	0	0	1	1	4									
35/65	0	0	0	0	0	0	0	0	1	0	1	2	3								
30/70	0	0	0	0	0	0	0	0	0	0	0	0	1	2							
25/75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1						
20/80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2					
15/85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1				
10/90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
5/95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0/100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Occurrences:	0	1	2	3	4	5

Figure 43: Heat map showing the frequency and occurrence of the incorrect ("indistinguishable" or "no recommendation") recommendations when using the previously determined optimal settings when comparing own-dyed cotton samples from different dye mixtures with LDA-own

A similar pattern is seen in the distribution of the erroneous recommendations using LDA-own as when using PCA-LDA, in that most errors are observed when the dye percentages in groups of fibres are similar; as opposed to being spread randomly across the data. However, it is obvious that LDA-own is more sensitive to differences in dye percentages than PCA-LDA for the reasons explained above (discrimination vs. variation during dimension reduction). Again, this suggests that the incorrect recommendations obtained were more due to the similarity in dye composition between the two groups of fibres being compared rather than the stringent upper/lower SPP threshold and/or exceedance proportion.

6.4.4 Own-dyed cotton – UV-vis range MSP

Up to this point, all of the MSP spectra have been obtained, and the subsequent MVA performed, using Vis range (380 – 710 nm) MSP only. In forensic casework, resources permitting, if two samples were found to be indistinguishable after microscopy and Vis range MSP, then the MSP range could be extended to include the UV range (i.e. reducing down to ~200 nm) [4,29,59,62,68,69,73,91,110,163]. In theory, by extending into the UV range, more information can be obtained that may allow for discrimination of samples by the fibre examiner.

In this study, down to ~280 nm was included as below this wavelength no useful features were observed in the MSP spectra; with only a large amount of noise being present. This would have in turn been introduced into the model classification system which may have resulted in poorer datasets being created during dimension reduction which may in turn negatively affect the subsequent classification accuracy.

Based on its previous utilisation in forensic fibre casework, it was anticipated that using UV-vis range MSP would increase the classification accuracy when comparing samples from different sources (i.e. dye mixtures). However, it was also hoped that the extra information provided in the UV region of the spectra may also allow samples from the same source to be more successfully recommended as being “indistinguishable” - increasing the classification accuracy in both scenarios. Because glass absorbs UV light, new samples had to be taken and mounted onto quartz slides, using glycerol as the mounting medium rather than phytohistol [73,118,119] rather than having to be simply read from the previously prepared slides (see “Methodology” section above and Chapter Two: “Materials and Methods”).

Samples were taken from the same five samples as used previously in an attempt to reduce as many additional variables as possible during the resampling and re-examination of the textile fibres.

6.4.4.1 Same Dye Mixture – UV-vis – PCA-LDA

When UV-vis range MSP data was used with groups of fibres that originated from the same dye mixture, lower recommendation accuracy was observed using UV-vis range MSP than Vis range MSP. When using PCA-LDA alongside UV-vis range MSP, 60% of the recommendations were incorrect; resulting in a recommendation accuracy of 40% (Figure 44). In contrast, when using Vis range MSP data with PCA-LDA, the overall classification accuracy was 96% (Figure 37).

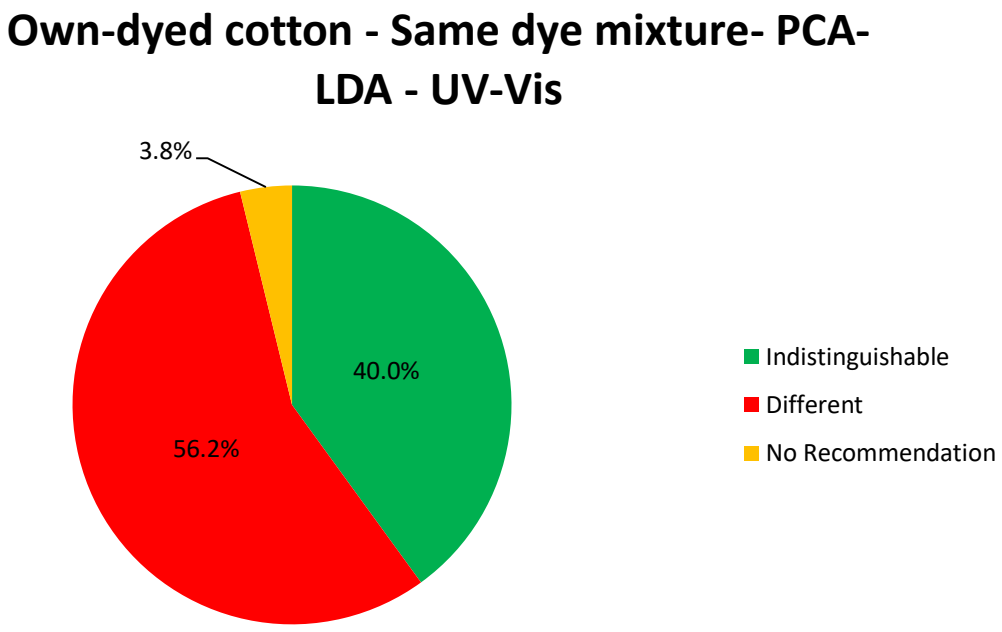

Figure 44: The average recommendation percentages when using own dyed cotton, SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibre= 10 when comparing groups of fibres from same dye mixtures with PCA-LDA and UV-vis range MSP

This drop in accuracy is believed to have been caused by the additional information being provided by the inclusion of the UV range in the MSP (moving from 380 nm as the lowest wavelength to 280 nm). This additional information, potentially alongside the different mounting medium and slide composition, affected the obtained spectra which then in turns results in a different dataset pre (and potentially post) dimension reduction. This new dataset appears to contain information less useful in the grouping of fibres that truly originate from the same dye mixture – resulting in the lower recommendation accuracy observed. This observation is not unexpected, as the inclusion of the UV range is often used as a method of increasing discrimination between two groups of fibres rather than looking to amplify similarities. This has also resulted in a large increase in false exclusions (i.e. giving an “excluded” recommendation when two groups of fibres

originate from the same source) when comparing fibres from the same dye mixture.

6.4.4.2 Different Dye Mixtures – UV-vis – PCA-LDA

In converse to the above situation, it was anticipated that the inclusion of the UV range into MSP would result in higher recommendation accuracy for the same logic above. This additional information present, would contain information useful when performing dimension reduction on datasets that originate from different dye mixtures. A recommendation accuracy of 85.0% was observed when using UV-vis MSP alongside PCA-LDA (Figure 45).

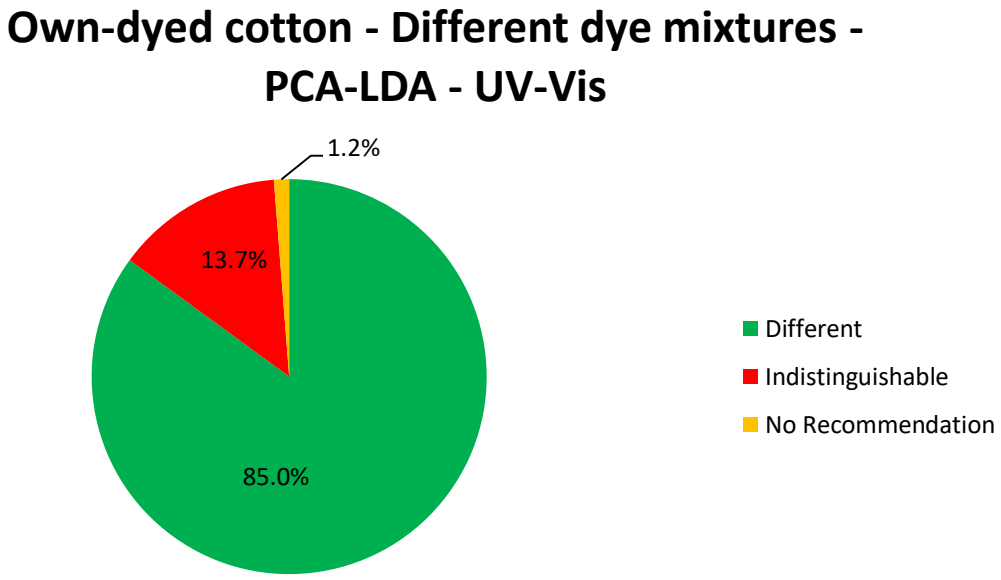

Figure 45: The average recommendation percentages when using own dyed cotton, SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibre= 10 when comparing groups of fibres from different dye mixtures with PCA-LDA and UV-vis range MSP

As anticipated, this 85.0% recommendation accuracy when utilising UV-vis range MSP was higher than the 68.4% classification accuracy observed when using Vis range MSP (Figure 38) - demonstrating that the inclusion of the UV range does

indeed provide useful information for discrimination. Therefore, it is not recommended that UV-vis range MSP is suitable for use alongside PCA-LDA; both because of the increase in false exclusions but also the increased material costs to the analysis in terms of equipment and consumables.

6.4.4.3 Same Dye Mixture – UV-vis – LDA-own

Although UV-vis range MSP was not suitable for application alongside PCA-LDA, LDA-own was also investigated as the utilised MVA approach to ensure that this combination did not provide optimal results.

When using Vis range MSP with LDA-own for fibres from the same dye mixture, a recommendation accuracy of 36% (was observed (Figure 41). However, when using LDA-own with UV-vis range MSP for fibres from the same dye mixture (Figure 46) only 2.4% recommendation accuracy was observed - by far the worst result observed throughout this research and obviously below any thresholds that would be required of any forensic investigation.

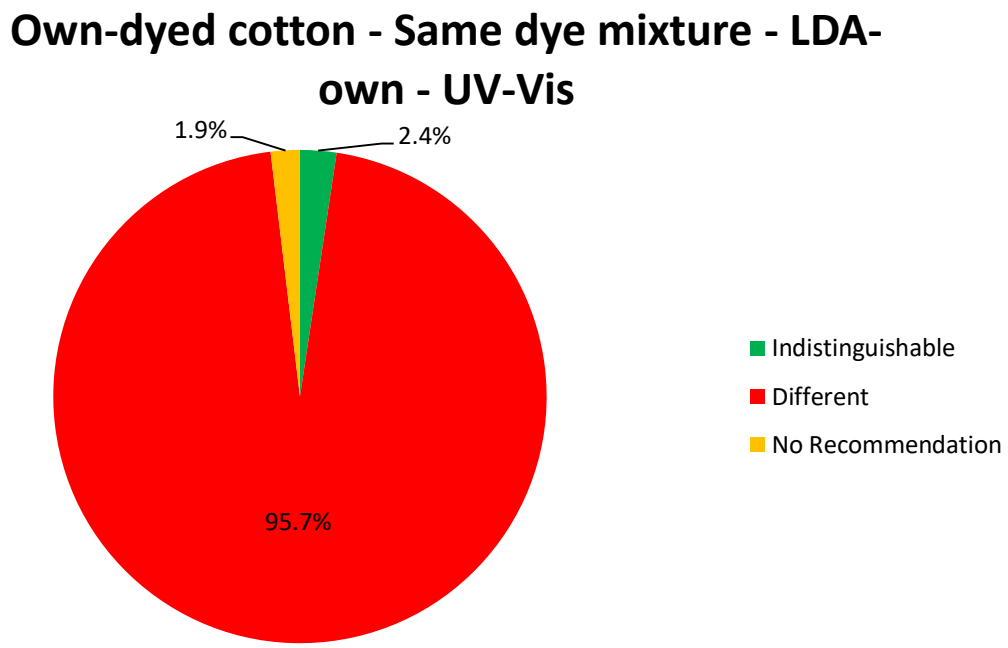

Figure 46: The average recommendation percentages when using own dyed cotton, SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibre= 10 when comparing groups of fibres from same dye mixtures with LDA-own and UV-vis range MSP

6.4.4.4 Different Dye Mixtures – UV-vis – LDA-own

To ensure completeness and fuller determination of limitations, the LDA-own approach was also investigated for use with UV-vis range MSP when considering fibres from different dye mixtures. When UV-vis range MSP was utilised alongside LDA-own the recommendation accuracy observed was 98.8% (Figure 47); higher than the 93.3% observed when using Vis range MSP (Figure 42) – again highlighting that the inclusion of the UV range when performed MSP can increase the discrimination power of the model classification system, but however results in an increase in the number of false exclusions that are made.

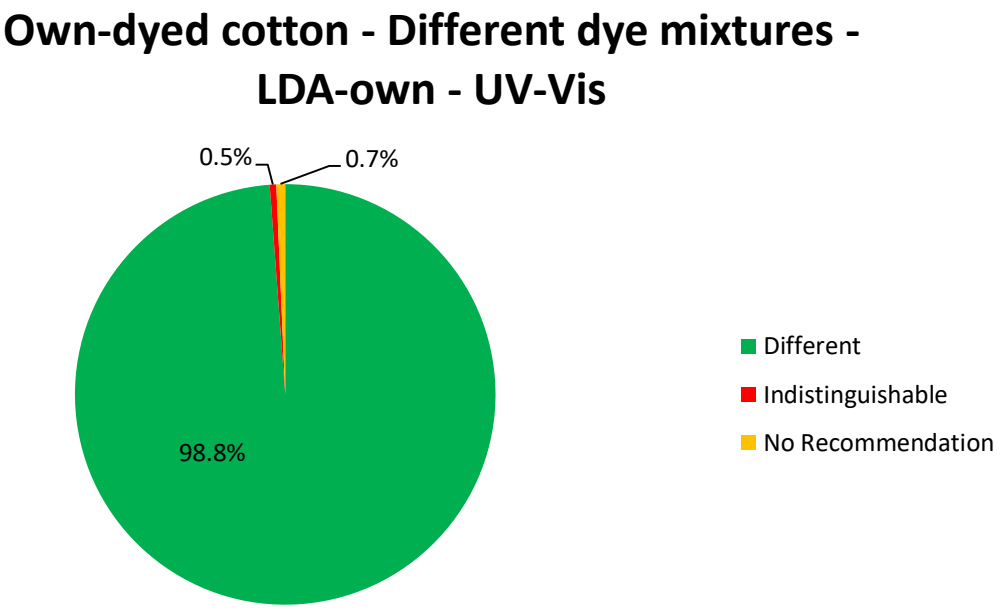

Figure 47: The average recommendation percentages when using own dyed cotton, SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibre= 10 when comparing groups of fibres from different dye mixtures with LDA-own and UV-vis range MSP

6.4.5 Summary – UV-vis range MSP

The results obtained and presented above using UV-vis range MSP suggest that caution should be used when applying UV-vis range MSP to either of the proposed MVA methods. As the MVA methods make their recommendation based solely on the MSP data provided to them, without being able to consider any previous results of examination, as would be the case with a fibre examiner. The potential exists to place too much emphasis on areas of discrimination in the UV range which may be detrimental should the groups of fibres have originated from the same source; resulting in an increased number of false exclusions i.e. incorrect "excluded" recommendations.

6.4.6 Single Fibre Scenarios

6.4.6.1 Same Source – Vis range MSP – PCA-LDA

A single fibre from each of the five samples in each dye mixture was selected. This single fibre had ten readings taken along its length to simulate a scenario where only a single fibre had been recovered for analysis and interpretation. These 10 readings were then each individually compared against the readings from 10 fibres for each of the five samples in the same dye mixture. This resulted in 105 comparisons being made between a single fibre and its true source, with the percentage of each recommendation shown in Figure 48.

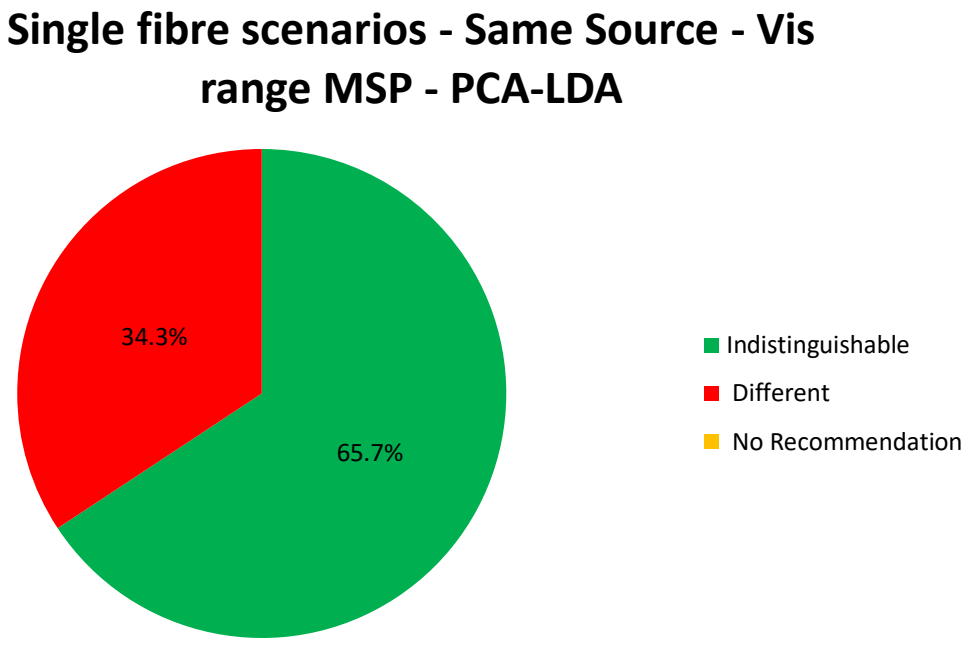

Figure 48: The average recommendation percentages when using single fibres from own dyed cotton. SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibres = 10, Number of Scans along single fibre = 10 when comparing groups of fibres from different dye mixtures with PCA-LDA and Vis range MSP

Figure 48 shows than a correct “indistinguishable” recommendation was given in 69 of the 105 instances (65.7%); down from the 96.0% observed previously when utilising own-dyed cotton samples where **10** fibres were available for both the

questioned and known groups of fibres (Figure 37) as opposed to a single fibre for the questioned group.

This drop in accuracy is likely due to only readings from a single fibre being taken into account when making the recommendation – as opposed to the previous situation utilising own dyed cotton where readings from 10 fibres from each sample were available. By only having a single fibre for analysis in the questioned group, it is much harder to ensure that the fibre in question is truly representative of its origin sample; whereas when 10 fibres are used it is much more likely to provide a representative sample – practice recommended by groups such as the European Textile and Hair Group in their fibre analysis guidelines for natural fibres such as cotton [41].

6.4.6.2 Different Dye Mixtures – Vis range MSP – PCA-LDA

To complement the above section, the 10 readings along the length of each single fibre from each (21) different dye percentage mixture were then compared against the readings from 10 fibres from all (20) different dye mixtures. This was repeated five times, using each of the (five) samples from each dye mixture; resulting in a total of 2100 (i.e. 21 x 20 x 5) comparisons being considered.

Overall, 81.7% of the comparisons (1716 of 2100) were correctly recommended to be “excluded” – with the average number of correct recommendations for each single fibre dye composition vs. the different mixtures shown in Figure 49. This 81.7% recommendation accuracy was higher than the 68.4% observed previously when utilising own-dyed cotton samples where 10 fibres were available for both the questioned and known groups of fibres (Figure 38). This increase in accuracy

is likely due to similar reasons as above relating to the representativeness of the fibre compared to the sample. With only one available fibre, it is much less likely that the single fibre *is* representative of the source and therefore is more likely to be recommended as being “excluded” when this may not in fact be the case (as seen in Figure 48).

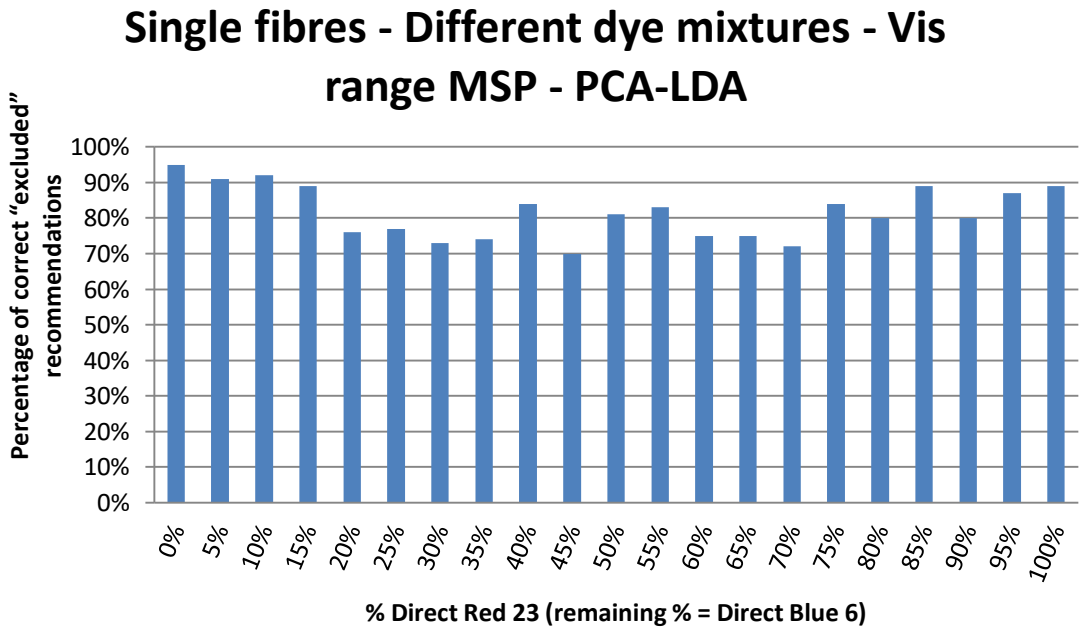

Figure 49: The average percentage of correct recommendations for each single fibre dye percentage when using own dyed cotton. SPP = 0.9999/0.0001, E.P. = 0.5, Number of fibres = 10, Number of Scans along single fibre = 10 when comparing groups of fibres from different dye mixtures with PCA-LDA and Vis range MSP

Figure 49 shows the average percentages of correct “exclusion” recommendations for each different dye mixture, when compared against the other dye mixtures. The overall average was 81.7%, with a standard deviation of 7.2%. The values range from the most successful (average 95% correct when comparing fibres dyed in 0% DR23 with 100% DB6 against the other fibres) and the least successful (average 70% correct when comparing fibres dyed in 45% DR23 with 55% DB6 against the other fibres).

Sauzier et al. took five MSP readings across 10 fibres from each source; resulting in 50 spectra in total for 10 blue acrylic sources. The first 45 spectra (i.e. MSP data from the first nine fibres from each blue acrylic source) were used as the "known" group and the final five spectra (i.e. the five spectra from the tenth blue acrylic source) as the "questioned" group. PCA followed by discriminant analysis was used to recommend if these comparisons were "inclusions or exclusions" (i.e. indistinguishable or distinguishable). Sauzier *et al.* reported 10 of 11 (~90.1%) correct "inclusion" results and 108 of 110 (~98.2%) "exclusion" results. In comparison, the optimal settings for the classification system when considering single fibre scenarios was 65.7% recommendation accuracy for "indistinguishable" samples (number of comparisons = 105), whereas when comparing a single fibre against fibres from different dye mixtures 81.7% recommendation accuracy was observed (number of comparisons = 2100). The proposed classification system therefore performed worse than the method proposed by Sauzier *et al.* when considering a single fibre scenario (and taking multiple readings from the same fibre) – however this research demonstrates a much larger sample size.

Sharma *et al.* [154] analysed dyes extracted from cotton and wool fibres through a variety of solvent systems using UV-vis spectrophotometry (200 – 800 nm). Visual comparison of the peaks obtained by spectrophotometry allowed ~84% of cotton fibres and ~94% of woollen fibres to be successfully discriminated. This number was reported to have increased to 100% for cotton fibres and ~98% for woollen fibres following the application of PCA and Welch's t-test. In comparison, when using UV-vis MSP with the proposed classification system, 85% recommendation accuracy was observed using PCA-LDA and 98.8% recommendation accuracy when using LDA-own.

Table 14: Summary of the accuracy of both MVA approaches when using Vis-Range MSP with the previously determined optimal settings for the model classification system

MVA Approach	Recommendation Accuracy – **Same** Source - Vis Range MSP – using previously determined optimal settings	Recommendation Accuracy – **Different** Source - Vis Range MSP – using previously determined optimal settings
PCA-LDA	96%	68.4%
LDA-own	36%	93.3%

Table 15:Summary of the accuracy of both MVA approaches when using UV-vis and Vis-Range MSP with the previously determined optimal settings for the model classification system

MVA Approach	**Recommendation Accuracy – Same Source - using previously determined optimal settings**		**Recommendation Accuracy – Different Source - using previously determined optimal settings**	
	Vis MSP	**UV-vis MSP**	**Vis MSP**	**UV-vis MSP**
PCA-LDA	96%	40%	68.4%	85.0%
LDA-own	36%	2.4%	93.3%	98.8%

Sharma *et al.* only examined eleven cotton fibres and fifteen woollen fibres – an obvious sample size issue. Additionally, the use of dye extraction from fibres prior to analysis constitutes a destructive approach – meaning that the fibres will no longer be able to be examined by other means. So, although the proposed MVA approach tended to perform worse than that demonstrated by Sharma *et al.* it is however better from an evidence maximisation standpoint – meaning that the

evidence is not exposed to destructive methods; allowing for future analysis to be performed which may increase the discrimination. Furthermore, Sharma *et al.* only considered the discrimination of fibres , and not the ability of the proposed method to determine if two groups of fibres that truly could have originated from the same source are not falsely excluded – therefore failing to address the fundamental question covered by this research.

6.5 Conclusion

Experiments were performed using own-dyed cotton where the percentage of dye between samples varied by 5% (in terms of composition of DR23 and DB6) to create the most visually and spectrally similar samples examined to date. The use of UV-vis MSP data was also examined to determine if this would increase recommendation accuracy for both scenarios: a) when groups of fibres originated from same sources (dye mixtures) and therefore should be recommended as being “indistinguishable”, and b) when groups of fibres originate from the different dye mixtures and should therefore recommended as being “excluded”. Single fibre scenarios were also considered, wherein instead of 10 fibres per group being available for both the known and questioned groups, only a single fibre was available for the questioned group, and to compensate, 10 readings were taken along the length of a single fibre.

When using Vis range MSP, PCA-LDA had 96% recommendation accuracy when recommending groups of fibres from the same dye mixture to be “indistinguishable”. However, this accuracy decreased to 68.4% when comparing fibres from different dye mixtures, which should have been recommended as being “excluded” The majority of the erroneous recommendations occurred when the difference in dye percentage were similar, as to be expected.

LDA-own demonstrated greater accuracy when making recommendations on fibres from different dye mixtures, with greater sensitivity than PCA-LDA. This was evidenced by 93.3% recommendation accuracy in this scenario. However, when making recommendations on fibres that originated from the same dye mixtures, LDA-own demonstrated a classification accuracy of 36%. The results from the two MVA approaches are summarised in Table 14.

Therefore, for the own-dyed cotton experiments using Vis range MSP, neither PCA-LDA nor LDA-own showed suitably high classification accuracy for *both* scenarios using these challenging comparisons.

The above experiments were then repeated using UV-vis range MSP data as opposed to just Vis range MSP. The inclusion of UV range data meant that more information was available for the subsequent MVA classification. It was hoped that this additional information could aid with discrimination of groups of fibres that originated from different sources, but also allow for more similarities to be present in the underlying data that would increase the recommendation accuracy of groups of fibres from the same dye mixture.

When UV range MSP data was included, PCA-LDA demonstrated an increased 85.0% classification accuracy for discriminating fibres from different sources – but a substantially decreased 40% classification accuracy for recommending when fibres originated from the same source. Similarly LDA-own demonstrated an increased 98.8% classification accuracy for discriminating fibres from different sources – but a substantially decreased 2.4% classification accuracy for recommending when fibres originated from the same source.

Overall, while the hypothesis that UV-vis range MSP would provide useful information to aid with discrimination of groups of cotton fibres that originated from different sources was true, it was found that this additional information resulting in a substantial increase in the number of false exclusions with considering groups of fibres that originated from the same dye mixture.

Finally, the model classification system (Vis Range MSP, PCA-LDA, previously determined optimal settings) was tested using single fibres – mimicking a scenario where only a single fibre has been recovered for analysis. To remedy this, 10 MSP readings were taken along the length of the single fibre, before comparing these to both readings of 10 fibres from the same dye mixture as well as different dye mixtures. When comparing a single fibre against fibres from the same dye mixture, 65.7% recommendation accuracy was observed, whereas when compared against fibres from different dye mixtures 81.7% recommendation accuracy was observed.

This represents one of the most challenging situations facing a fibre examiner, and similarly the proposed model classification system also found these challenging. The results suggest that based on the limited ability of the model classification system to successfully recommend when two fibres truly originated from the same source (false exclusions). It also shows that the increased discrimination between samples is more likely due to differences being detected due to unrepresentative data from a single fibre vs. the known group, the application of the model classification system does not seem appropriate when only a single fibre is available for analysis.

7. Conclusions

The aim of this research was to determine if multivariate analysis (MVA), specifically principal component analysis (PCA) and linear discriminant analysis (LDA), could successfully be applied to textile fibre evidence to objectively classify if two groups of microspectrophotometry (MSP) spectra from fibres are indistinguishable or distinguishable. The answer to this is yes - but with some limitations which are discussed below.

In order to achieve this aim, the following objectives were explored:

"Outline the requirements for an "ideal" classification system"

With regards to outlining the requirements of an ideal classification system, it was decided that the method should:

i. Utilise a probabilistic approach
ii. Require minimal user input
iii. Be robust

By meeting the above, it allowed for the proposed classification system to be more objective and free from bias then the currently employed methodologies of textile fibre analysis; while remaining applicable to forensic casework scenarios.

“Determine the optimal settings to allow for high accuracy when using fibres from clearly (visually and spectrally) distinguishable sources, as well as fibres from the same source and should therefore be indistinguishable.”

Extensive testing of various self-predictive probability (SPP), exceedance proportion (E.P.) and number of fibres per group thresholds were examined with both MVA approaches; PCA-LDA LDA-own. Regardless of which MVA approach was utilised, the following were found to be the optimal settings to be used:

- Upper/Lower SPP = 0.9999/0.0001
- E.P. = 0.5
- Number of Fibres per Group = 10

These settings, combined with the appropriate MVA approach, resulted in suitable classification accuracy for both indistinguishable and distinguishable groups of fibres from cotton and acrylic sources.

“Determine if these optimal settings can be successfully applied to the most common blocks of colour encountered in forensic science i.e. where fibres will be less obviously visually and spectrally distinguishable.”

These previously determined optimal settings were then provided with more casework like samples and scenarios – wherein the groups of fibres originated from the same broad colour/fibre type; referred to as a colour block. The previously determined optimal settings again proved to be suitable, demonstrating 100% accuracy when making recommendations on groups of fibres from the same source (indistinguishable) and an average of 90% accuracy when making recommendations on groups of fibres from different sources (distinguishable).

“Determine the limits of sensitivity of the proposed methods when examining fibres of the same material, with differing proportions of dye present in each bulk sample.”

Very small changes in dye percentage between samples are where the proposed technique struggled the most with its discrimination. Although 96% accuracy was observed when making recommendations on groups of fibres from the same dye mixture, the accuracy dropped to 68.4% when attempting to differentiate fibres from different dye mixtures. Most of the erroneous recommendations occurred when the dyed fibres were within ~20% similarity in dye composition – illustrating the limitation of discrimination with the technique. Further research would be required to determine the limit of discrimination of the human eye system and a proposed suite of analysis for comparison.

“Propose a methodology when working with single fibres – one of the most challenging situations for a fibre examiner.”

A solution to single fibres situations was attempted, whereby ten readings along the length of a single fibre were used to make comparisons against a group of ten fibres. This proved to be the most challenging, with 65.7% classification accuracy when comparing a single fibre to a group of fibres from the same source, and 81.7% classification accuracy when comparing a single fibre to a group of fibres from a different source. The former is particularly disappointing – suggesting that having only a single fibre for analysis results in not enough variation being captured from the single fibre to be able to confidently place it within the control group.

7.1 Implications to forensic casework

This work demonstrated that the proposed classification could see successful application to forensic casework. However, the following limitations are currently in place:

- The number of fibres available for analysis from both groups must be ten
- The system has been most comprehensively tested for cotton fibres. When working with cotton fibres, the PCA-LDA approach should be used.
- For fibres of a very similar dye composition, caution should be used when any “excluded” outcome is given – suggesting that the groups of fibres are distinguishable and therefore not from the same source.
- This classification system is not currently recommended when a single fibre is being compared
- Further research is required to have increased confidence in findings with regards to fibre type outside of cotton – although some preliminary data is presented with for acrylic fibres. When approaching acrylic fibres, it is recommended to use the LDA-own approach as the acrylic fibres tend to be more uniform and appear to benefit from the increased discrimination focus of this approach.

7.2 Future work

This research has contributed to the gap of knowledge regarding the application of MVA to textile fibre evidence – however further work can always be done and improvements can be made. This would include the application of machine learning as an expansion to MVA.

The research could be performed and validated on a wider variety of fibre types to determine if the proposed classification system holds true over a wider range of fibre types. It is also possible that the classification system can be combined with Bayes Theorem to quantify the likelihood that a fibre originated from the same source *given* the classification (i.e. moving from sub-source to source level).

It was also discussed as to whether or not a combined approach to the MVA methods would be beneficial, given that PCA-LDA was more accurate in some scenarios and LDA-own in others. It is suggested that it may be possible to create some form of consensus between the two that could improve the confidence in the classification, or at minimum reduce any erroneous results by incorporating a larger acceptance of uncertainty.

Finally, the proposed settings could be fine-tuned and experimented further; with the potential to maximise the classification accuracy, while increasing the application of the proposed technique to forensic casework. This would be best if suitable settings could be demonstrated utilising smaller number of fibres per groups (i.e. allowing the system to work with fewer than 10 fibres per group).

8. Glossary

AU - Absorbance Units - A unit of measurement for absorbance by equipment such as MSP

DB6 - Direct Blue 6 - A direct dye used to dye cotton

DR23 - Direct Red 23 - A direct dye used to dye cotton

E.P. - Exceedance Proportion - The proportion of recommendations from the MVA approach required to give an overall recommendation

ENFSI - European Network of Forensic Science Providers - Network of forensic providers for information sharing

ETHG - European Textile and Hair Group - Forensic group covering hair and other textiles

HPLC - High Performance Liquid Chromatography - A separation technique

IR - Infrared - Refers to a region of the electromagnetic spectrum with wavlength > ~800nm

LDA - Linear Discriminant Analysis - A multivariate analysis technique

LDA-own - The application of linear discriminant analysis as an MVA approach, WITHOUT the prior use of dimension reduction by principal component analysis

LOOCV - Leave One Out Cross Validation - A method within multiavariate analysis where one piece of data is removed, the model is trained with the remaining data, and then the removed data tested against the model.

mL - millilitres - unit of volume measurement

MSP - Microspectrophotometry - An objective technique for measuring absorbance of light in various substrates such as fibres

MVA - Multivariate Analysis - Statistical methods that consider more than one variable at a time

nm - nonameters - unit of measurement for distance, used to describe wavelengths

PCA - Principal component analysis - A statistical technique often used to look for linear combinations of variables, resulting in dimension reduction

PCA-LDA - The application of linear discriminant analysis as an MVA approach, AFTER the prior use of dimension reduction by principal component analysis

PCs - Principle Components - Linear combinations of original variables of a dataset

SOP - Standard Operating Proceedure - A document outlining the typical use of a piece of equipment or technique

SPP - Self-Predictive Probability - The probability of the MVA model assigning a fibre back to its original group

SWGMAT - Scientific Working Group for Materials Analysis - A scientific group that studies fibres, paints, glass etc.

TLC - Thin Layer Chromatography - A separation technique

UV - Ultraviolet - Refers to area of the electromagnetic spectrum with a wavelength < ~400 nm

UV-vis - Ultraviolet -visible range - MSP approach that utilised the wavelength range from 280 - 710 nm

Vis - Visible range MSP - MSP approach that utilised the wavelength range from 380-710 nm

9. Appendix 1 – MSP SOP document

Operation of the J&M TIDAS MSP400 UV-vis Range Microspectrophotometer

Start-Up

1. If using the system in UV-vis Mode, Turn on UV source BEFORE the PC and MSP.
2. Acquire – Initialise (MSP shutters activate ‘Green ‘Busy’ light illuminates
3. Acquire-camera-initialise
4. Acquire-camera-start (viewing screen activates)

Visible Range Configuration

- Place sample on microscope stage.

  (*N.B. For Visible range only measurements, sample can be mounted using glass slides, coverslips and a mountant such as DPX).*

- Use x10 objective and using the camera window, locate sample on slide and focus. Switch to x40 Plan-Neoflar objective and refocus.
- Acquisition – Settings;
  - Scan Type: Absorbance
  - Start Wavelength:
  - End Wavelength: 710
  - Int. Time: 600ms
  - No. Averages: 10
  - Binning: 1
  - Dark Current: selected.

UV-vis Configuration

- Place sample on microscope stage.

  (*N.B. For UV-vis range measurements, sample must be mounted on a quartz slide using glycerol and covered with a quartz coverslip).*

- Use x10 objective and using the camera window, locate sample on slide and focus. Switch to x40 UV objective and refocus.

- Acquisition – Settings;
    - Scan Type: Absorbance
    - Start Wavelength: 240nm
    - End Wavelength: 710nm
    - Int. Time: 600ms
    - No. Averages: 10
    - Binning: 1
    - Dark Current: selected.

Background Spectrum

1. Move measurement window off fibre
2. Deselect dark current from Acquisition-settings
3. Select Scan type to 'Counts' in Acquisition – Settings
4. Select Acquisition – Monitor: system now continually refreshes background spectrum. Adjust sub-stage condenser rack until a maximum count is achieved without disrupting the microscope slide. Typical values achieved should be > 25000 (for UV).
5. Press Esc to exit monitor mode.
6. Reselect acquisition parameters as above: ***The system is now configured for UV-vis Range.***
7. Acquisition – **background spectrum;** the system now acquires the background spectrum.

Sample Spectrum

1. Move measurement area in camera window onto the fibre – ensuring that the fibre is orientated 'north-south'.
2. Acquisition – Take Spectrum; the system now prompts for sample identifiers and description. Press OK: Spectrum is acquired.
3. File – Save As: Save spectrum with appropriate filename into desired folder.

   For synthetic fibres a minimum of 5 control fibres should be analysed and saved. For naturally occurring fibres a minimum of 10 should be analysed .

(*N.B. These are general guidelines. It may be appropriate to analyse more fibres and/ or more than one reading along the length of a fibre, depending on the inter- and intra sample variation*)

10. Appendix 2 – R Scripts

10.1 computeSPP

```
require(MASS)
require(psych)
##############################################################
#    load the two functions (a) PCA+LDA and (b) LDA
##############################################################
source('C:/Users/Laptopuser/Documents/R/R files and outputs/Own Dyed/scripts/PCA_LDA_comb.R')
source('C:/Users/Laptopuser/Documents/R/R files and outputs/Own Dyed/scripts/LDA_own_tol_1e-6_18-7-16.R')
#####################################################################
user inputs
#####################################################################
workdir <- 'C:/Users/Laptopuser/Documents/R/R files and outputs/Own Dyed/'
setwd(workdir)
fibre.type <- 'cotton'
choose.fibres <- 20   # number of fibres to be used in total (over all samples)
                # e.g., if 20 specified with two samples, then each sample contains 10 fibres
normalised <- FALSE
analysis.type <- 'pairwise'    #  pairwise = all combinations of two colours
                        #  single    = one single colour (the sole-colour setings in the paper)

choose.colour <- # the chosen sole colour
#'yellow'
#'orange'
```

```
#'tangerine'
#'bronze'
#'brightred'
#'cardinal'
#'grenadine'
#'lightberry'
#'violet'
#'purple'
#'bluejewel'
#'olympicblue'
#'mediumnavy'
#'windsorblue'
#'seafoam'
#'emeraldgreen'
#'paddygreen'
#'mediumavocado'
#'tan'
#'mediumbrown'
#'coffee'
#'nickel'
#'black'

nsets <- 50    #  number of random allocations to be used for the sole-colour settings
if (analysis.type!='single') choose.colour <- NULL
#####################
#  load data
```

```
#####################
if (fibre.type=='acrylic') {
   temp <- read.csv('csv files/acrylic new 20 (21-40) NN CSV.csv',header=TRUE)
   if (normalised) temp <- read.csv('23 acrylic normalised data CSV.csv',header=TRUE)
}
if (fibre.type=='cotton') {
#       temp <- read.csv('data/cotton/Blue Cotton Colour Block - Med Blue NN CSV.csv',header=TRUE)
#    if (normalised) temp <- read.csv('data/cotton/Blue Cotton Colour Block - Med Blue N CSV.csv',header=TRUE)
#    names(temp)[which(names(temp)=='group')] <- 'colourname'
   temp <- read.csv('csv files/sample 5 NN CSV 1stDer 21ptSmth.csv',header=TRUE)
   if (normalised) temp <- read.csv('Cotton (23) N CSV.csv',header=TRUE)
}
w.id <- 3:406
####  positions of the wavelengths
wavelength <- sapply(names(temp)[w.id],function(x){as.numeric(sub('w','',x))})
g <- as.character(temp$colourname)
id <- which(g=='')
if (length(id)>0) g <- g[-c(id)]
#if (!is.null(choose.colour)) g <- g[grep(choose.colour,g)]
colours <- unique(g)
ncolours <- length(colours)
#  a matrix with all wavelengths (row: samples and column: wavelengths)
all.data <- matrix(unlist(temp[c(w.id)]),ncol=length(w.id),byrow=FALSE)
```

```
if (length(id)>0) all.data <-
matrix(unlist(temp[c(w.id)]),ncol=length(w.id),byrow=FALSE)[-c(id),]
temp.nfibres <- unique(table(g))
if (length(temp.nfibres)>1) stop('some colours have different numbers of fibres in ')
if (analysis.type=='pairwise') {
  npcs <- 15   #   maximum number of PCs to be used in PCA+LDA
  nsamples <- 2    #   number of sources in each set
  nfibres <- choose.fibres/nsamples   #  number of fibres per source
  total.samples <- nfibres*nsamples   #  total number of fibres in each set
  if (nfibres<10) npcs <- 9  # there is little variability beyond the 9th PC (LDA will
complain!)
  #  constructing exhaustive pairs
  pr <- colour.settings <- combn(ncolours,2)
  colour.settings[1,] <- colours[pr[1,]]
  colour.settings[2,] <- colours[pr[2,]]
  colour.settings <- t(colour.settings)
  nsets <- ncol(pr)
}
if (analysis.type=='blocks') {    ####   not used   #####
  npcs <- 30   #   maximum number of PCs to be used in PCA+LDA
  nsamples <- 4
  total.samples <- nfibres*nsamples
  blues <- c('bluejewel','olympicblue','mediumnavy','windsorblue')
  purples <- c('purple','violet','grenadine','lightberry')
  reds <- c('grenadine','lightberry','brightred','cardinal')
  greens <- c('seafoam','emeraldgreen','paddygreen','mediumavacado')
  browns <- c('tan','mediumbrown','coffee','bronze')
```

```
  yellows <- c('yellow','orange','tangerine','bronze')
  colour.settings <- array('',c(6,4))
  colour.settings[1,] <- blues
  colour.settings[2,] <- purples
  colour.settings[3,] <- reds
  colour.settings[4,] <- greens
  colour.settings[5,] <- browns
  colour.settings[6,] <- yellows
  nsets <- nrow(colour.settings)
}
if (analysis.type=='all') {
  npcs <- 30   #   maximum number of PCs to be used in PCA+LDA
  nsamples <- ncolours
  total.samples <- nfibres*nsamples
  colour.settings <- matrix(colours,nrow=1)
  nsets <- 1
}
if (analysis.type=='single') {
  colours <- choose.colour
  ncolours <- 1
  npcs <- 15   #   maximum number of PCs to be used in PCA+LDA
  nsamples <- 2
  nfibres <- choose.fibres/2
  total.samples <- nfibres*nsamples
  if (nfibres<10) npcs <- 9  # there is little variability beyond the 9th PC (LDA will
complain!)
  temp <- combn(1:total.samples,nfibres)
```

```
 splits <- temp[,seq(1,ncol(temp),ncol(temp)/nsets)]
 colour.settings <- matrix(rep(colours,2*nsets),nrow=nsets)
 for (i in 1:nsamples) colour.settings[,i] <-
sapply(1:nsets,function(x){paste(colours,i,sep='')})
}
#############################################################
#  set up storage arrays
#############################################################
#    for PCA+LDA
pca.lda.all.probs <- array(0,c(nsets,npcs,total.samples,nsamples))
pca.lda.prob.truth <- array(0,c(nsets,npcs,total.samples))
pca.kaiser <- rep(0,nsets)   #  positions of the last PC with eigenvalue>1
pca.lda.SPP.by.sample <- array(0,c(nsets,npcs,nsamples))
#pca.sdev <- array(0,c(nsets,npcs))  #  variance partition
#    for LDA
lda.all.probs <- array(0,c(nsets,total.samples,nsamples))
lda.prob.truth<- array(0,c(nsets,total.samples))
lda.SPP.by.sample <- array(0,c(nsets,nsamples))
sample.names <- colours
if (analysis.type=='single') sample.names <-
sapply(1:nsamples,function(x){paste(colours,x,sep='')})
if (analysis.type=='pairwise') sample.names <- c('colour1','colour2') ####    see
colour.settings for the colour pairs
colnames(lda.SPP.by.sample) <- sample.names
dimnames(pca.lda.SPP.by.sample)[[3]] <- sample.names

#############################################################
```

```
#  perform PCA-LDA and LDA-own for each colour set
#############################################################
for (iset in 1:nsets) {
  #   select fibres in the chosen colour set
  if (analysis.type=='single') {
    set.ids <- which(g==colours)[1:choose.fibres]  # select fibre samples according
to choose.fibres
  }
  if (analysis.type=='pairwise') {
    set.ids <- c(sapply(colour.settings[iset,],function(x){which(g==x)[1:nfibres]}))
  }
  wl.mat <- all.data[set.ids,]
  gp <- g[set.ids]
  if (analysis.type=='single') {
    gp <- sapply(1:total.samples,function(x){paste(colours,2,sep='')})
    gp[splits[,iset]] <- sapply(1:nfibres,function(x){paste(colours,1,sep='')})
  }
  for (ipc in 1:npcs) {
    ############    PCA + LDA
    res <- pca.lda.comb(wl.mat,gp,ipc,CV=TRUE)
    pca.kaiser[iset] <- res$kaiser
    #        pca.sdev[iset,] <- res$sdev
    pca.lda.prob.truth[iset,ipc,] <- res$pred[,'ProbTruth']
    pca.lda.all.probs[iset,ipc,,] <- as.matrix(res$pred[,colour.settings[iset,]])
    if   (analysis.type=='single')   pca.lda.SPP.by.sample[iset,ipc,sample.names]   <-
res$SPP.by.sample[sample.names]
```

```
  if (analysis.type=='pairwise') pca.lda.SPP.by.sample[iset,ipc,] <-
res$SPP.by.sample[colour.settings[iset,]]
 }
 ##########  LDA on its own
 res <- lda.own(wl.mat,gp,CV=TRUE)
 lda.prob.truth[iset,] <- res$pred[,'ProbTruth']
 lda.all.probs[iset,,] <- as.matrix(res$pred[,colour.settings[iset,]])
 if (analysis.type=='single') lda.SPP.by.sample[iset,sample.names] <-
res$SPP.by.sample[sample.names]
 if (analysis.type=='pairwise') lda.SPP.by.sample[iset,] <-
res$SPP.by.sample[colour.settings[iset,]]
 print(paste('  finished colour pair ',iset,sep=''))
}
###################################
####  save results
save.file <-
paste('results/',fibre.type,'/',choose.fibres,'fibres_',analysis.type,'_normalised.RDat
a',sep='')
if (!normalised) save.file <-
paste('results/',fibre.type,'/',choose.fibres,'fibres_',analysis.type,'_unnormalised.RD
ata',sep='')
if (analysis.type=='single')
 save.file <- paste('results/',fibre.type,'/',analysis.type,'_',colours,'_'
          ,total.samples,'fibres_',nsets,'splits_unnormalised.RData',sep='')
save(file=save.file,pca.lda.prob.truth,pca.lda.all.probs,lda.prob.truth,lda.all.probs,c
olour.settings,pca.kaiser
   ,pca.lda.SPP.by.sample,lda.SPP.by.sample)
```

10.2 PCA_LDA_comb

```
#########################################################################
####   function to perform PCA + LDA
####   first use PCA to reduce wavelengths then LDA to classify fibres
####   examples:
####                              res        <-        pca.lda.comb(wl.mat[-c(10),],gp[-
c(10)],'kaiser',dat.pred=wl.mat[10,],CV=FALSE)
####    res <- pca.lda.comb(wl.mat,gp,5,CV=TRUE)
#########################################################################
#############

pca.lda.comb                                                                    <-
function(dat,grouping,npcs,single.PC=NULL,center=TRUE,scale.=TRUE,rotate=N
ULL,dat.pred=NULL,CV=TRUE) {
   #  =======
   #    input:
   #      dat:       an array of size n-by-m with training measures (n=number of fibres
and m=wavelengths)
   #      grouping  the known grouping of the training objects
   #      npcs       number of PCs used in the subsequent LDA
   #                 if npcs='kaiser', PCs will be selected using Kaiser Criterion
   #       single.PC only one chosen PC is used for LDA (e.g., if single.PC=3, then
use only the 3rd PC in LDA)
   #                 default=NULL, using all the first n PCs defined by npcs
   #      center     logical if measurements are centred by removing the mean
   #      scale.     logical if measurements are scaled to have variance 1
   #      rotate     method to rotate the PCs
```

```
    #      dat.pred  a matrix with measurements for prediction (same format as dat)
    #      CV        if a leave-one-out CV to be carried out
    #  =======
    #    output:
    #      kaiser    number of PCs chosen using the Kaiser Criterion
    #      sdev      SD of the PC scores (~ square root of the eigenvalues)
    #      pred      posterior probabilities
    ##############################################################

    group.names <- unique(grouping)
    ngroups <- length(group.names)

    if (!is.null(single.PC)) npcs <- 1  #  only one PC is used in LDA

    ##### ====  Step 1: perform PCA to combine wavelengths for both training and
predict data
    #   putting both training set and predicting set (if exists) together
    all.dat <- dat
    if (!is.null(dat.pred)) all.dat <- rbind(dat,dat.pred)
    n <- nrow(all.dat)
    if (is.null(rotate)) {
        pcfit <- prcomp(all.dat,center=center,scale.=scale.)
    }
    ####   various outputs from PCA
    #   number of PCs required based on Kaiser Criterion
    kaiser <- which(pcfit$sdev<1)[1] - 1
    #   SD of the PCs (~square root of the eigenvalue)
```

```
sdev <- pcfit$sdev
##### ====  Step 2: perform LDA on the selected PCs
if (npcs=='kaiser') npcs <- kaiser
npcs <- as.numeric(npcs)
scores <- pcfit$x[,1:npcs]
if (!is.null(single.PC)) scores <- pcfit$x[,single.PC]  #  single PC used

pred <- array(0,c(n,ngroups));colnames(pred) <- group.names

####   perform prediction using LDA
pred.ids <- n
if (CV) pred.ids <- 1:n
npreds <- length(pred.ids)
prob.truth <- rep(NULL,npreds)
for (ipred in 1:npreds) {
   pred.id <- pred.ids[ipred]
   train.ids <- (1:n)[-c(pred.id)]
   if (npcs==1) {
      train.scores <- matrix(scores[train.ids],ncol=1)
      pred.scores <- matrix(scores[pred.id],ncol=1)
   }
      if (npcs>1) {
      train.scores <- scores[train.ids,]
      pred.scores <- scores[pred.id,]
   }
   lda.fits <- lda(train.scores,grouping=grouping[train.ids],cv=FALSE)
   if (CV) {
```

```
            pred[pred.id,] <- predict(lda.fits,pred.scores)$posterior[1,group.names]
            prob.truth[pred.id] <- pred[pred.id,grouping[pred.id]]
        } else {
            pred <- predict(lda.fits,pred.scores)$posterior[1,group.names]
        }
    }
    ####   preparing output
    if (!is.matrix(pred)) pred <- matrix(pred,nrow=1)
    pred <- data.frame(pred)
    names(pred) <- group.names
    if (CV) {
        pred$Truth <- grouping
        pred$ProbTruth <- prob.truth
        ####   average probability of prediciting the truth (i.e. SPP) by sample
        SPP.by.sample                                                    <-
sapply(group.names,function(x){mean(pred$ProbTruth[which(pred$Truth==x)])})
    }
    pred$chosenPCs <- rep(npcs,npreds)
    if (npcs==kaiser) chosenPCs <- rep('kaiser',npreds)

return(list(kaiser=kaiser,sdev=sdev,pred=pred,SPP.by.sample=SPP.by.sample))
}
```

10.3 LDA_own

```
#####################################################################
####   function to perform LDA on its own
####   examples
####      res <- lda.own(wl.mat[-c(10),],gp[-c(10)],dat.pred=wl.mat[10,],CV=FALSE)
####      res <- lda.own(wl.mat,gp,CV=TRUE)
#####################################################################
#############
lda.own                                                              <-
function(dat,grouping,center=TRUE,scale.=TRUE,dat.pred=NULL,CV=TRUE,tol=1
.0e-6) {
   #  =======
   #    input:
   #      dat:       an array of size n-by-m with training measures (n=number of fibres
and m=wavelengths)
   #      grouping  the known grouping of the training objects
   #      center    logical if measurements are centred by removing the mean
   #      scale.    logical if measurements are scaled to have variance 1
   #      dat.pred  a matrix with measurements for prediction (same format as dat)
   #      CV        if a leave-one-out CV to be carried out
   #  =======
   #    output:
   #      pred      posterior probabilities
   ############################################################
   group.names <- unique(grouping)
   ngroups <- length(group.names)
```

```
scores <- dat
if (!is.null(dat.pred)) scores <- rbind(dat,dat.pred)
n <- nrow(scores)

pred <- array(0,c(n,ngroups));colnames(pred) <- group.names
####   perform prediction using LDA
pred.ids <- n
if (CV) pred.ids <- 1:n
npreds <- length(pred.ids)
prob.truth <- rep(NULL,npreds)
for (ipred in 1:npreds) {
   pred.id <- pred.ids[ipred]
   train.ids <- (1:n)[-c(pred.id)]
   if (npcs==1) {
      train.scores <- matrix(scores[train.ids],ncol=1)
      pred.scores <- matrix(scores[pred.id],ncol=1)
   }
   if (npcs>1) {
      train.scores <- scores[train.ids,]
      pred.scores <- scores[pred.id,]
   }
   lda.fits <- lda(train.scores,grouping=grouping[train.ids],cv=FALSE,tol=tol)
   if (CV) {
      pred[pred.id,] <- predict(lda.fits,pred.scores)$posterior[1,group.names]
      prob.truth[pred.id] <- pred[pred.id,grouping[pred.id]]
   } else {
      pred <- predict(lda.fits,pred.scores)$posterior[1,group.names]
```

```
    }
  }
  ####   preparing output
  if (!is.matrix(pred)) pred <- matrix(pred,nrow=1)
  pred <- data.frame(pred)
  names(pred) <- group.names
  if (CV) {
    pred$Truth <- grouping
    pred$ProbTruth <- prob.truth
    ####   average probability of prediciting the truth (i.e. SPP) by sample
    SPP.by.sample                                                    <-
sapply(group.names,function(x){mean(pred$ProbTruth[which(pred$Truth==x)])})
  }
  return(list(pred=pred,SPP.by.sample=SPP.by.sample))
```

10.4 summary_decision_rule

```
#######################################################################
####   this script summarises the results from applying a decision rule to make
####   recommendation about the origin of two recovered fibre samples
#######################################################################
rm(list=ls())
require(MASS)
require(psych)

#######################################################################
####  user inputs
#######################################################################
workdir <- 'C:/Users/Laptopuser/Documents/R/R files and outputs/Own Dyed/'
setwd(workdir)

method.type <- 'lda-own'
#method.type <- 'pca-lda-kaiser'

fibre.type <- 'cotton'
#fibre.type <- 'acrylic'
choose.fibres <- 20   # number of fibres to be used in total (over all samples)
                # e.g., if 20 specified with two samples, then each sample contains
10 fibres
total.samples <- choose.fibres
analysis.type <- 'pairwise'    #  pairwise = all combinations of two colours
                    #   single    = one single colour (the sole-colour setings in the
paper)
```

```
nsets <- 50

normalised <- FALSE


colours = c('0_1','5_1','10_1','15_1','20_1','25_1','30_1','35_1','40_1','45_1','50_1'
        ,'55_1','60_1','65_1','70_1','75_1','80_1','85_1','90_1','95_1','100_1',
        '0_2','5_2','10_2','15_2','20_2','25_2','30_2','35_2','40_2','45_2','50_2'
        ,'55_2','60_2','65_2','70_2','75_2','80_2','85_2','90_2','95_2','100_2',
        '0_3','5_3','10_3','15_3','20_3','25_3','30_3','35_3','40_3','45_3','50_3'
        ,'55_3','60_3','65_3','70_3','75_3','80_3','85_3','90_3','95_3','100_3',
        '0_4','5_4','10_4','15_4','20_4','25_4','30_4','35_4','40_4','45_4','50_4'
        ,'55_4','60_4','65_4','70_4','75_4','80_4','85_4','90_4','95_4','100_4',
        '0_5','5_5','10_5','15_5','20_5','25_5','30_5','35_5','40_5','45_5','50_5'
        ,'55_5','60_5','65_5','70_5','75_5','80_5','85_5','90_5','95_5','100_5')
#colours = c('black', 'coffee', 'lightberry', 'olympicblue', 'paddygreen')
#colours <-  c('yellow','orange','tangerine','bronze','brightred','cardinal',
          #'grenadine','lightberry','violet','purple','bluejewel','olympicblue',
          #'mediumnavy','windsorblue','seafoam','emeraldgreen','paddygreen',
          #'mediumavocado','tan','mediumbrown','coffee','nickel','black')
#c('black','bluejewel','brightred','bronze','cardinal','coffee','emeraldgreen','grenadine
','lightberry','mediumavocado','mediumbrown','mediumnavy','nickel','olympicblue','o
range','paddygreen','purple','seafoam','tan','tangerine','violet','windsorblue','yellow')
thresholds <- c(0.0001,0.9999)
#thresholds <- c(0.00001,0.99999)
#thresholds <- c(0.01,0.99)
same.cut <- different.cut <- 0.5

############################################################
```

```
#    load functions related to the decision rule
############################################################
source('C:/Users/Laptopuser/Documents/R/R files and outputs/Own
Dyed/scripts/decision_rules.R')

if (analysis.type=='pairwise') colours <- 'pairwise'
ncolours <- length(colours)
recm <- array(0,c(ncolours,3))
colnames(recm) <- c('indistinguishable','different','no recommendation')
rownames(recm) <- colours
par(mfrow=c(1,4))
for (colour in colours) {
   file <-
paste('results/',fibre.type,'/',choose.fibres,'fibres_',analysis.type,'_normalised.RDat
a',sep='')
   if (!normalised) file <-
paste('results/',fibre.type,'/',choose.fibres,'fibres_',analysis.type,'_unnormalised.RD
ata',sep='')
   if (analysis.type=='single')
     file <-
paste('results/',fibre.type,'/',analysis.type,'_',colour,'_',total.samples,'fibres_',nsets,'
splits_unnormalised.RData',sep='')
   load(file)
   if (method.type=='lda-own') {
     s <-
t(apply(lda.prob.truth,1,function(x){decision.rules(x,thresholds=thresholds)$table}))
   }
```

```
    if (method.type=='pca-lda-kaiser') {
      ####   select results according to the kaiser criterion
      d <- dim(lda.prob.truth)
      res <- array(0,c(d[1],d[2]))
      for (i in 1:length(pca.kaiser)) res[i,] <- pca.lda.prob.truth[i,pca.kaiser[i],]
      s <- t(apply(res,1,function(x){decision.rules(x,thresholds=thresholds)$table}))
    }
    plot(density(s[,1]),xlim=c(0,1),main=colour,xlab='% in each of the three
categories')
    for (i in 2:3) lines(density(s[,i]),col=i,lty=1)
    ####   recommendation for each set
    for (i in 1:nrow(s)) {
        if (s[i,'confident']>different.cut) {
            recm[colour,'different'] <- recm[colour,'different'] + 1
        } else if (s[i,'uncertain']>same.cut) {
            recm[colour,'indistinguishable'] <- recm[colour,'indistinguishable'] + 1
        } else {
            recm[colour,'no recommendation'] <- recm[colour,'no recommendation'] + 1
        }
    }
    abline(v=same.cut,col=2,lty=3)
    abline(v=different.cut,col=1,lty=3)
}
legend('topright',legend=c('% confident','% no recommendation','%
misclassified'),col=1:3,lty=1)
analysis.type;method.type
recm
```

```
apply(recm/50,2,max)

apply(recm/50,2,median)

apply(recm/50,2,mean)


if (analysis.type=='pairwise') recm/sum(recm)*100
```

10.5 decision_rules

```
classify.fibre <- function(spp,thresholds=c(0.01,0.99)) {
    #    classify one fibre at a time
    decision <- 'uncertain'
    if (spp>thresholds[2]) decision <- 'confident'
    if (spp<thresholds[1]) decision <- 'misclassified'
    return(decision)
}

summary.classify.fibre <- function(cls) {
    groups <- c('confident','uncertain','misclassified')
    sm <- sapply(groups,function(x){length(which(cls==x))})
    return(sm)
}

decision.rules                                                    <-
function(spp,thresholds=c(0.01,0.99),confident.cut=0.9,uncertain.cut=0.6) {
    nfibres <- length(spp)
    cls <- sapply(spp,function(x){classify.fibre(x,thresholds)})
    s <- summary.classify.fibre(cls)/nfibres
    decision <- 'not sure'
    if (s['confident'] > confident.cut) decision <- 'different'
    if (s['uncertain'] > uncertain.cut) decision <- 'same'
    return(list(classification=cls,table=s,decision=decision))
}
```

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